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(71) Applicant: NPS PHARMACEUTICALS, INC. [US/US]; Suite 241, 420 Chipeta Way, Salt Lake City, UT 84108-1256 (US).

(72) Inventors: FULLER, Forrest, H.; 1401 Military Way, Salt Lake City, UT 84103 (US). KRAPCHO, Karen, J.; 3340 San Raphael Avenue, Salt Lake City, UT 84109 (US). HAMMERLAND, Lance, G.; 3201 South 400 East, Bountiful, UT 84010 (US).

(74) Agents: WARBURG, Richard, J. et al.; Lyon & Lyon, First Interstate World Center, Suite 4700, 633 West Fifth Street, Los Angeles, CA 90071 (US).

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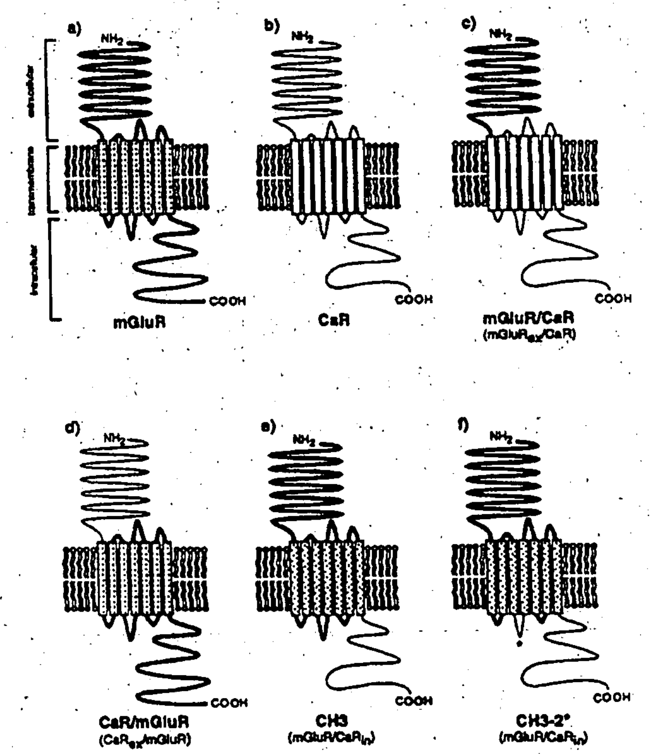
(57) Abstract

The present invention provides chimeric receptors. The chimeric receptors comprise at least one region homologous to a region of a metabotropic glutamate receptor and at least one region homologous to a region of a calcium receptor. The invention also includes methods of preparing such chimeric receptors, and methods of using such receptors to identify and characterize compounds which modulate the activity of metabotropic glutamate receptors or calcium receptors. The invention also relates to compounds and methods for modulating metabotropic glutamate receptor activity and binding to metabotropic glutamate receptors. Modulation of metabotropic glutamate receptor activity can be used for different purposes such as treating neurological disorders and diseases, inducing an analgesic effect, cognition enhancement, and inducing a muscle-relaxant effect.



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CHIMERIC RECEPTORS AND METHODS FOR IDENTIFYING COMPOUNDS ACTIVE AT METABOTROPIC GLUTAMATE RECEPTORS AND THE USE OF SUCH COMPOUNDS IN THE TREATMENT OF NEUROLOGICAL DISORDERS AND DISEASES

FIELD OF THE INVENTION

The present invention relates to chimeric receptors containing one or more regions homologous to a metabotropic glutamate receptor and one or more regions homologous to a calcium receptor.

BACKGROUND OF THE INVENTION

The following description provides a summary of information relevant to the present invention. It is not an admission that any of the information provided herein is prior art to the presently claimed invention, nor that

any of the publications specifically or implicitly referenced are prior art to that invention.

Glutamate is the major excitatory neurotransmitter in the mammalian brain. Glutamate produces its effects on central neurons by binding to and thereby activating cell surface receptors. These receptors have been subdivided into two major classes, the ionotropic and metabotropic glutamate receptors, based on the structural features of the receptor proteins, the means by which the receptors transduce signals into the cell, and pharmacological profiles.

The ionotropic glutamate receptors (iGluRs) are ligand-gated ion channels that, upon binding glutamate, open to allow the selective influx of certain monovalent and divalent cations, thereby depolarizing the cell membrane. In addition, certain iGluRs with relatively high calcium permeability can activate a variety of calcium-dependent intracellular processes. These receptors are multisubunit protein complexes that may be homomeric or heteromeric in nature. The various iGluR subunits all share common structural motifs, including a relatively large amino-terminal extracellular domain (ECD), followed by a multiple transmembrane domain (TMD) comprising two membrane-spanning regions (TMs), a second smaller intracellular loop, and a third TM, before terminating with an intracellular carboxy-terminal domain (CT). Historically the iGluRs were first subdivided pharmacologically into three classes based on preferential activation by the agonists alpha-amino-3-hydroxy-5-methyl-

isoxazole-4-propionic acid (AMPA), kainate (KA), and N-methyl-D-aspartate (NMDA). Later, molecular cloning studies coupled with additional pharmacological studies revealed a greater diversity of iGluRs, in that multiple subtypes of AMPA, KA and NMDA receptors are expressed in the mammalian CNS (Hollman and Heinemann, *Ann. Rev. Neurosci*. 7:31, 1994).

The metabotropic glutamate receptors (mGluRs) are G protein-coupled receptors capable of activating a variety of intracellular second messenger systems following the binding of glutamate or other potent agonists including quisqualate and 1-aminocyclopentane-1,3-dicarboxylic acid (*trans*-ACPD) (Schoepp *et al*., *Trends Pharmacol. Sci*. *11*:508, 1990; Schoepp and Conn, *Trends Pharmacol. Sci*. *14*:13, 1993).

Activation of different metabotropic glutamate receptor subtypes *in situ* elicits one or more of the following responses: activation of phospholipase C, increases in phosphoinositide (PI) hydrolysis, intracellular calcium release, activation of phospholipase D, activation or inhibition of adenylyl cyclase, increases and decreases in the formation of cyclic adenosine monophosphate (cAMP), activation of guanylyl cyclase, increases in the formation of cyclic guanosine monophosphate (cGMP), activation of phospholipase A_2, increases in arachidonic acid release, and increases or decreases in the activity of voltage- and ligand-gated ion channels (Schoepp and Conn, *Trends Pharmacol. Sci*. 14:13,

1993; Schoepp, *Neurochem. Int.* 24:439, 1994; Pin and Duvoisin, *Neuropharmacology* 34:1, 1995).

Thus far, eight distinct mGluR subtypes have been isolated via molecular cloning, and named mGluR1 to mGluR8 according to the order in which they were discovered (Nakanishi, *Neuron* 13:1031, 1994, Pin and Duvoisin, *Neuropharmacology* 34:1, 1995; Knopfel et al., *J. Med. Chem.* 38:1417, 1995). Further diversity occurs through the expression of alternatively spliced forms of certain mGluR subtypes (Pin et al., *PNAS* 89:10331, 1992; Minakami et al., *BBRC* 199:1136, 1994). All of the mGluRs are structurally similar, in that they are single subunit membrane proteins possessing a large amino-terminal extracellular domain (ECD) followed by seven putative transmembrane domain (7TMD) comprising seven putative membrane spanning helices connected by three intracellular and three extracellular loops, and an intracellular carboxy-terminal domain of variable length (cytoplasmic tail) (CT) (*see*, Schematic Figure 1a).

The eight mGluRs have been subdivided into three groups based on amino acid sequence identities, the second messenger systems they utilize, and pharmacological characteristics (Nakanishi, *Neuron* 13:1031, 1994; Pine and Duvoisin, *Neuropharmacology* 34:1, 1995; Knopfel et al., *J. Med. Chem.* 38:1417, 1995). The amino acid identity between mGluRs within a given group is approximately 70% but drops to about 40% between mGluRs in different groups. For mGluRs in the same group, this relatedness is roughly

paralleled by similarities in signal transduction mechanisms and pharmacological characteristics.

The Group I mGluRs comprise mGluR1, mGluR5 and their alternatively spliced variants. The binding of agonists to these receptors results in the activation of phospholipase C and the subsequent mobilization of intracellular calcium. For example, *Xenopus* oocytes expressing recombinant mGluR1 receptors have been utilized to demonstrate this effect indirectly by electrophysiological means (Masu et al., *Nature* 349:760, 1991; Pin et al., *PNAS* 89:10331, 1992). Similar results were achieved with oocytes expressing recombinant mGluR5 receptors (Abe et al., *J. Biol. Chem.* 267:13361, 1992; Minakami et al., *BBRC* 199:1136, 1994). Alternatively, agonist activation of recombinant mGluR1 receptors expressed in Chinese hamster ovary (CHO) cells stimulated PI hydrolysis, cAMP formation, and arachidonic acid release as measured by standard biochemical assays (Aramori and Nakanishi, *Neuron* 8:757, 1992). In comparison, activation of mGluR5 receptors expressed in CHO cells stimulated PI hydrolysis and subsequent intracellular calcium transients but no stimulation of cAMP formation or arachidonic acid release was observed (Abe et al., *J. Biol. Chem.* 267:13361, 1992). The agonist potency profile for Group I mGluRs is quisqualate > glutamate = ibotenate > (2S,1'S,2'S)-2-carboxycyclopropyl)glycine (L-CCG-I) > (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD). Quisqualate is relatively selective for Group I receptors,

as compared to Group II and Group III mGluRs, but it also potently activates ionotropic AMPA receptors (Pin and Duvoisin, Neuropharmacology, 34:1, Knopfel et al., *J. Med. Chem.* 38:1417, 1995).

The Group II mGluRs include mGluR2 and mGluR3. Activation of these receptors as expressed in CHO cells inhibits adenylyl cyclase activity via the inhibitory G protein, G_i, in a pertussis toxin-sensitive fashion (Tanabe et al., *Neuron* 8:169, 1992; Tanabe et al., *Neurosci.* 13:1372, 1993). The agonist potency profile for Group II receptors is L-CCG-I>glutamate>ACPD>ibotenate>quisqualate. Preliminary studies suggest that L-CCG-I and (2S,1'R,2'R,3'R)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV) are both relatively selective agonists for the Group II receptors (Knopfel et al., *J. Med. Chem.* 38:1417, 1995).

The Group III mGluRs include mGluR4, mGluR6, mGluR7 and mGluR8. Like the Group II receptors these mGluRs are negatively coupled to adenylate cyclase to inhibit intracellular cAMP accumulation in a pertussis toxin-sensitive fashion when expressed in CHO cells (Tanabe et al., *J. Neurosci.* 13:1372, 1993; Nakajima et al., *J. Biol. Chem.* 268:11868, 1993; Okamoto et al., *J. Biol. Chem.* 269:1231, 1994; Duvoisin et al., *J. Neurosci.* 15:3075, 1995). As a group, their agonist potency profile is (S)-2-amino-4-phosphonobutyric acid (L-AP4)>glutamate>ACPD>quisqualate, but mGluR8 may differ slightly with glutamate being more potent than L-AP4 (Knopfel et al., *J. Med. Chem.* 38:1417, 1995; Duvoisin et

al., *J. Neurosci.* 15:3075, 1995). Both L-AP4 and (S)-serine-O-phosphate (L-SOP) are relatively selective agonists for the Group III receptors.

Finally, the eight mGluR subtypes have unique patterns of expression within the mammalian CNS that in many instances are overlapping (Masu et al., *Nature* 349:760, 1991; Martin et al., *Neuron* 9:259, 1992; Ohishi et al., *Neurosci.* 53:1009, 1993; Tanabe et al., J. Neurosci. 13:1372; Ohishi et al., *Neuron* 13:55, 1994, Abe et al., *J. Biol. Chem.* 267:13361, 1992; Nakajima et al., *J. Biol. Chem.* 268:11868, 1993; Okamoto et al., *J. Biol. Chem.* 269:1231, 1994; Duvoisin et al., *J. Neurosci.* 15:3075, 1995). As a result certain neurons may express only one particular mGluR subtype, while other neurons may express multiple subtypes that may be localized to similar and/or different locations on the cell (i.e., postsynaptic dendrites and/or cell bodies versus presynaptic axon terminals). Therefore, the functional consequences of mGluR activation on a given neuron will depend on the particular mGluRs being expressed; the receptors' affinities for glutamate and the concentrations of glutamate the cell is exposed to; the signal transduction pathways activated by the receptors; and the locations of the receptors on the cell. A further level of complexity may be introduced by multiple interactions between mGluR expressing neurons in a given brain region. As a result of these complexities, and the lack of subtype-specific mGluR agonists and antagonists, the roles of particular mGluRs in physiological and pathophysiological processes

affecting neuronal function are not well defined. Still, work with the available agonists and antagonists have yielded some general insights about the Group I mGluRs as compared to the Group II and Group III mGluRs.

Attempts at elucidating the physiological roles of Group I mGluRs suggest that activation of these receptors elicits neuronal excitation. Various studies have demonstrated that ACPD can produce postsynaptic excitation upon application to neurons in the hippocampus, cerebral cortex, cerebellum, and thalamus as well as other brain regions. Evidence indicates that this excitation is due to direct activation of postsynaptic mGluRs, but it has also been suggested to be mediated by activation of presynaptic mGluRs resulting in increased neurotransmitter release (Baskys, *Trends Pharmacol. Sci.* 15:92, 1992; Schoepp, *Neurochem. Int.* 24:439, 1994; Pin and Duvoisin, *Neuropharmacology* 34:1). Pharmacological experiments implicate Group I mGluRs as the mediators of this excitation. The effect of ACPD can be reproduced by low concentrations of quisqualate in the presence of iGluR antagonists (Hu and Storm, *Brain Res.* 568:339, 1991; Greene et al. *Eur. J. Pharmacol.* 226:279, 1992), and two phenylglycine compounds known to activate mGluR1, (S)-3-hydroxyphenylglycine ((S)-3HPG) and (S)-3,5-dihydroxyphenylglycine ((S)-DHPG), also produce the excitation (Watkins and Collingridge, *Trends Pharmacol. Sci.* 15:333, 1994). In addition, the excitation can be blocked by (S)-4-carboxyphenylglycine ((S)-4CPG), (S)-4-carboxy-3-hydroxyphenylglycine ((S)-4C3HPG) and (+)-alpha-

methyl-4-carboxyphenylglycine ((+)-MCPG), compounds known to be mGluR1 antagonists (Eaton et al., *Eur. J. Pharmacol.* 244:195, 1993; Watkins and Collingridge, *Trends Pharmacol. Sci.* 15:333, 1994).

Other studies examining the physiological roles of mGluRs indicate that activation of presynaptic mGluRs can block both excitatory and inhibitory synaptic transmission by inhibiting neurotransmitter release (Pin and Duvoisin, *Neuropharmacology* 34:1). Presynaptic blockade of excitatory synaptic transmission by ACPD has been observed on neurons in the visual cortex, cerebellum, hippocampus, striatum and amygdala (Pin et al., *Curr. Drugs: Neurodegenerative Disorders* 1:111, 1993), while similar blockade of inhibitory synaptic transmission has been demonstrated in the striatum and olfactory bulb (Calabresi et al., *Neurosci. Lett.* 139:41, 1992; Hayashi et al., *Nature* 366:687, 1993). Multiple pieces of evidence suggest that Group II mGluRs mediate this presynaptic inhibition. Group II mGluRs are strongly coupled to inhibition of adenylyl cyclase, like alpha$_2$-adrenergic and SHT_{1A}-serotonergic receptors which are known to mediate presynaptic inhibition of neurotransmitter release in other neurons. The inhibitory effects of ACPD can also be mimicked by L-CCG-I and DCG-IV, which are selective agonists at Group II mGluRs (Hayashi et al., *Nature* 366:687, 1993; Jane et al., *Br. J. Pharmacol.* 112:809, 1994). Moreover, it has been demonstrated that activation of mGluR2 can strongly inhibit presynaptic, N-type calcium channel activity when the receptor is expressed in

sympathetic neurons (Ikeda et al., *Neuron* 14:1029, 1995), and inactivation of these channels is known to inhibit neurotransmitter release. Finally, it has been observed that L-CCG-I, at concentrations selective for Group II mGluRs, inhibits the depolarization-evoked release of ^{3}H-aspartate from rat striatal slices (Lombardi et al., *Br. J. Pharmacol.* 110:1407, 1993). Evidence for physiological effects of Group II mGluR activation at the postsynaptic level is limited. However, one study suggests that postsynaptic actions of L-CCG-I can inhibit NMDA receptor activation in cultured mesencephalic neurons (Ambrosini et al., *Mol. Pharmacol.* 47:1057, 1995).

Physiological studies have demonstrated that L-AP4 can also inhibit excitatory synaptic transmission on a variety of CNS neurons. Included are neurons in the cortex, hippocampus, amygdala, olfactory bulb and spinal cord (Koerner and Johnson, Excitatory Amino Acid Receptors: Design of Agonists and Antagonists p. 308, 1992; Pin et al., *Curr. Drugs: Neurodegenerative Disorders* 1:111, 1993). The accumulated evidence indicates that the inhibition is mediated by activation of presynaptic mGluRs. Since the effects of L-AP4 can be mimicked by L-SOP, and these two agonists are selective for Group III mGluRs, members of this mGluR group are implicated as the mediators of the presynaptic inhibition (Schoepp, *Neurochem. Int.* 24:439, 1994; Pin and Duvoisin, *Neuropharmacology* 34:1). In olfactory bulb neurons it has been demonstrated that L-AP4 activation of mGluRs inhibits presynaptic calcium currents (Trombley and Westbrook, *J.*

Neurosci. 12:2043, 1992). It is therefore likely that the mechanism of presynaptic inhibition produced by activation of Group III mGluRs is similar to that for Group II mGluRs, i.e., blockade of N-type calcium channels and inhibition of neurotransmitter release. L-AP4 is also known to act postsynaptically to hyperpolarize ON bipolar cells in the retina. It has been suggested that this action may be due to activation of a mGluR, which is coupled to the cGMP phosphodiesterase in these cells (Schoepp, *Neurochem. Int.* 24:439, 1994; Pin and Duvoisin, *Neuropharmacology* 34:1).

Metabotropic glutamate receptor activation studies using agonists, antagonists and recombinant vertebrate cell lines expressing mGluRs have been used to evaluate the cellular effects of the stimulation and the inhibition of different metabotropic glutamate receptors. For example, agonist stimulation of mGluR1 expressed in *Xenopus* oocytes demonstrated coupling of receptor activation to mobilization of intracellular calcium as assessed indirectly using electrophysiology techniques (Masu *et al.*, *Nature* *349*:760-765, 1991). Agonist stimulation of mGluR1 expressed in CHO cells stimulated PI hydrolysis, cAMP formation and arachidonic acid release (Aramori and Nakanishi, *Neuron* *8*:757-765, 1992). Agonist stimulation of mGluR5 expressed in CHO cells also stimulated PI hydrolysis which was shown to be associated with a transient increase in cytosolic calcium as assessed by loading cells with the fluorescent calcium chelator fura-2 (Abe *et al.*, *J. Biol. Chem.* *267*:13361-13368, 1992).

Agonist-induced activation of mGluR1 and mGluR5 induced PI hydrolysis in CHO cells was not antagonized by AP3 and AP4, which are both antagonists of glutamate-stimulated PI hydrolysis *in situ* (Nicoletti *et al.*, *Proc. Natl. Acad. Sci. USA 833*:1931-1935, 1986; Schoepp and Johnson, *J. Neurochem. 53*:273-278, 1989). Agonist stimulation of CHO cells expressing mGluR2 (Tanabe *et al.*, *Neuron 8*:169-179, 1992) or mGluR7 (Okamoto *et al.*, *J. Biol. Chem. 269*:1231-1236, 1994) resulted in receptor-mediated inhibition of cAMP formation and also confirmed the ligand specificity previously observed *in situ*. Studies using agonists were also carried out in conjunction with site-directed mutagenesis to reveal specific amino acids playing important roles in glutamate binding (O'Hara *et al.*, *Neuron 11*:41-52, 1993).

Metabotropic glutamate receptors (mGluRs) have been implicated in a variety of neurological pathologies including stroke, head trauma, spinal cord injury, epilepsy, ischemia, hypoglycemia, anoxia, and neurodegenerative diseases such as Alzheimer's disease (Schoepp and Conn, *Trends Pharmacol. Sci. 14*:13, 1993; Cunningham *et al.*, *Life Sci.* 54: 135, 1994; Pin et al., *Neuropharmacology* 34:1, 1995; Knopfel et al., *J. Med. Chem.* 38:1417, 1995;). A role for metabotropic glutamate receptors in nociception and analgesia has also been demonstrated (Meller *et al.*, *Neuroreport 4*:879, 1993). Metabotropic glutamate receptors have also been shown to be required for the induction of hippocampal long-term potentiation and cerebellar long-term depression (Bashir

et al., *Nature 363*:347, 1993; Bortolotto *et al.*, *Nature 368*:740, 1994; Aiba et. al. *Cell* 79: 365 and *Cell* 79: 377, 1994).

Metabotropic glutamate receptor agonists have been reported to have effects on various physiological activities. For example, *trans*-ACPD was reported to possess both proconvulsant and anticonvulsant effects (Zheng and Gallagher, *Neurosci. Lett. 125*:147, 1991; Sacaan and Schoepp, *Neurosci. Lett. 139*:77, 1992; Taschenberger *et al.*, *Neuroreport 3*:629, 1992; Sheardown, *Neuroreport* 3:916, 1992), and neuroprotective effects *in vitro* and *in vivo* (Pizzi *et al.*, *J. Neurochem. 61*:683, 1993; Koh *et al.*, *Proc. Natl. Acad. Sci. USA 88*:9431, 1991; Birrell *et al.*, *Neuropharmacol.* 32:1351, 1993; Siliprandi *et al.*, *Eur. J. Pharmacol. 219*:173, 1992; Chiamulera *et al.*, *Eur. J. Pharmacol. 216*:335, 1992). The metabotropic glutamate receptor antagonist L-AP3 was shown to protect against hypoxic injury *in vitro* (Opitz and Reymann, *Neuroreport 2*:455, 1991). A subsequent study reported that *trans*-ACPD produced neuroprotection which was antagonized by L-AP3 (Opitz and Reymann, *Neuropharmacol.* 32:103, 1993). (S)-4C3HPG was shown to protect against audiogenic seizures in DBA/2 mice (Thomasen et al., *J. Neurochem.* 62:2492, 1994). Other modulatory effects expected of metabotropic glutamate receptor modulators include synaptic transmission, neuronal death, neuronal development, synaptic plasticity, spatial learning, olfactory memory, central control of cardiac activity, waking, control of movements, and

control of vestibulo ocular reflex (for reviews, see Nakanishi, *Neuron* 13:1031-37, 1994; Pin et al., *Neuropharmacology* 34:1, 1995; Knopfel et al., *J. Med. Chem.* 38:1417, 1995).

The structures of mGluR-active molecules currently known in the art are limited to amino acids which appear to act by binding at the glutamate binding site (Pin, et al, *Neuropharmacology* 34:1, 1995; Knopfel et al., *J. Med. Chem.* 38:1418). This limits the range of pharmacological properties and potential therapeutic utilities of such compounds. Furthermore, the range of pharmacological specificities associated with these mGluR-active molecules does not allow for complete discrimination between different subtypes of metabotropic glutamate receptors (Pin et al., *Neuropharmacology* 34:1, 1995 and Knopfel et al., *J. Med. Chem.* 38:1418). Rapid progress in the field of mGluR-active molecules cannot be made until more potent and more selective mGluR agonists, antagonists and modulators are discovered (Pin et al., *Neuropharmacology* 34:1, 1995; Knopfel et al., *J. Med. Chem.* 38:1418). Indeed, no mGluR-active molecules are presently under clinical development. High throughput functional screening of compounds and compound libraries using cell lines expressing individual mGluRs represents an important approach to identifying such novel compounds (Knopfel et al., *J. Med. Chem.* 38:1418).

Several laboratories have constructed cell lines expressing metabotropic glutamate receptors which appear to function appropriately (Abe et al., *J. Biol. Chem.*

267:13361, 1992; Tanabe et al., *Neuron* 8:169, 1992; Aramori and Nakanishi, *Neuron* 8:757, 1992, Nakanishi, *Science* 258:597, 1992; Thomsen et al., *Brain Res.* 619:22, 1992; Thomsen et al., *Eur. J. Pharmacol.* 227:361, 1992; O'Hara et al., *Neuron* 11:41, 1993; Nakjima et al., *J. Biol. Chem.* 268:11868, 1993; Tanabe et al., *J. Neurosci.* 13:1372, 1993; Saugstad et al., *Mol. Pharmacol.* 45:367, 1994; Okamoto et al., *J. Biol. Chem.* 269:1231, 1994; Gabellini et al., *Neurochem. Int.* 24:533, 1994; Lin et al., *Soc. Neurosci. Abstr.* 20:468, 1994; Flor et al., *Soc. Neurosci. Abstr.* 20:468, 1994; Flor et al., *Neuropharmacology* 34:149, 1994). Other reports have noted that expression of functional mGluR expressing cell lines is not predictable. For example, Tanabe et al., (*Neuron* 8:169, 1992) were unable to demonstrate functional expression of mGluR3 and mGluR4, and noted difficulty obtaining expression of native mGluR1 in CHO cells. Gabellini et al., (*Neurochem. Int.* 24:533, 1994) also noted difficulties with mGluR1 expression in HEK 293 cells and it is possible that some of these difficulties may be due to desensitization characteristics of these receptors. Furthermore, screening methodologies useful for identification of compounds active at Class I mGluRs are not readily amenable to identification of compounds active at class II and III mGluRs and *vice versa* due to the differences in second messenger coupling. Finally, mGluRs have been noted to rapidly desensitize upon agonist stimulation which may adversely affect the viability of

glutamate, asparagine and, to a lesser extent, methionine; the nonpolar aliphatic amino acids glycine, alanine, valine, isoleucine, and leucine (however, because of size, glycine and alanine are more closely related and valine, isoleucine and leucine are more closely related); and the aromatic amino acids phenylalanine, tryptophan, and tyrosine. In addition, although classified in different categories, alanine, glycine, and serine seem to be interchangeable to some extent, and cysteine additionally fits into this group, or may be classified with the polar neutral amino acids.

While proline is a nonpolar neutral amino acid, its replacement represents difficulties because of its effects on conformation. Thus, substitutions by or for proline are not preferred, except when the same or similar conformational results can be obtained. The conformation conferring properties of proline residues may be obtained if one or more of these is substituted by hydroxyproline (Hyp).

Examples of modified amino acids include the following: altered neutral nonpolar amino acids such as ω-amino acids of the formula $H_2N(CH_2)_nCOOH$ where n is 2-6, sarcosine (Sar), t-butylalanine (t-BuAla), t-butylglycine (t-BuGly), N-methyl isoleucine (N-MeIle), and norleucine (Nleu); altered neutral aromatic amino acids such as phenylglycine; altered polar, but neutral amino acids such as citrulline (Cit) and methionine sulfoxide (MSO); altered neutral and nonpolar amino acids such as cyclohexyl alanine (Cha); altered acidic amino acids such

as cysteic acid (Cya); and altered basic amino acids such as ornithine (Orn).

Preferred derivatives have one or more amino acid alteration(s) which do not significantly affect the receptor activity of the related receptor protein. In regions of the receptor protein not necessary for receptor activity amino acids may be deleted, added or substituted with less risk of affecting activity. In regions required for receptor activity, amino acid alterations are less preferred as there is a greater risk of affecting receptor activity. Such alterations should be conservative alterations. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent.

Conserved regions tend to be more important for protein activity than non-conserved regions. Standard procedures can be used to determine the conserved and non-conserved regions important of receptor activity using *in vitro* mutagenesis techniques or deletion analyses and measuring receptor activity as described by the present disclosure.

Derivatives can be produced using standard chemical techniques and recombinant nucleic acid techniques. Modifications to a specific polypeptide may be deliberate, as through site-directed mutagenesis and amino acid substitution during solid-phase synthesis, or may be accidental such as through mutations in hosts which produce the polypeptide. Polypeptides including

derivatives can be obtained using standard techniques such as those described in Section I.G.2. *supra*, and by Sambrook *et al.*, *Molecular Cloning*, Cold Spring Harbor Laboratory Press (1989). For example, Chapter 15 of Sambrook describes procedures for site-directed mutagenesis of cloned DNA.

By "hyperalgesia" is meant an increased response to a stimulus that is normally painful.

By "minimal" is meant that any side effect of the drug is tolerated by an average individual, and thus that the drug can be used for therapy of the target disease or disorders. Such side effects are well known in the art. Preferably, minimal side effects are those which would be regarded by the FDA as tolerable for drug approval for a target disease or disorder.

By "modulate" is meant to cause an increase or decrease in an activity of a cellular receptor.

By "modulator" is meant a compound which modulates a receptor, including agonists, antagonists, allosteric modulators, and the like. Preferably, the modulator binds to the receptor.

By "muscle relaxant" is meant a compound that reduces muscular tension.

By "neuralgia" is meant pain in the distribution of a nerve or nerves.

By "neurodegenerative disease" is meant a neurological disease affecting cells of the central nervous system resulting in the progressive decrease in the ability of cells of the nervous system to function properly.

Examples of neurodegenerative diseases include Alzheimer's disease, Huntington's disease, and Parkinson's disease.

By "neurological disorder or disease" is meant a disorder or disease of the nervous system. Examples of neurological disorders and diseases include global and focal ischemic and hemorrhagic stroke, head trauma, spinal cord injury, hypoxia-induced nerve cell damage as in cardiac arrest or neonatal distress, and epilepsy.

By "neuroprotectant activity" is meant efficacy in treatment of the neurological disorders or diseases.

By "physically detectable means" is meant any means known to those of ordinary skill in the art to detect binding to or modulation of mGluR or CaR receptors, including the binding and screening methods described herein. Thus, for example, such means can include spectroscopic methods, chromatographic methods, competitive binding assays, and assays of a particular cellular function, as well as other techniques.

By "potent" is meant that the compound has an EC_{50} value (concentration which produces a half-maximal activation), or IC_{50} (concentration which produces half-maximal inhibition), or K_d (concentration which produces half-maximal binding) at a metabotropic glutamate receptor, with regard to one or more receptor activities, of less than 100 μM, more preferably less than 10 μM, and even more preferably less than 1 μM.

By "selective" is meant that the compound activates, inhibits activation and/or binds to a metabotropic glutamate receptor at a lower concentration than that at

which the compound activates, inhibits activation and/or binds to an ionotropic glutamate receptor. Preferably, the concentration difference is a 10-fold, more preferably 50-fold, and even more preferably 100-fold.

By "therapeutically effective amount" is meant an amount of a compound which produces the desired therapeutic effect in a patient. For example, in reference to a disease or disorder, it is the amount which reduces to some extent one or more symptoms of the disease or disorder, and returns to normal, either partially or completely, physiological or biochemical parameters associated or causative of the disease or disorder. When used to therapeutically treat a patient it is an amount expected to be between 0.1 mg/kg to 100 mg/kg, preferably less than 50 mg/kg, more preferably less than 10 mg/kg, more preferably less than 1 mg/kg. Preferably, the amount provides an effective concentration at a metabotropic glutamate receptor of about 1 nM to 10 μM of the compound. The amount of compound depend on its EC_{50} (IC_{50} in the case of an antagonist) and on the age, size, and disease associated with the patient.

II. Techniques

A. Chimeric Receptors and General Approach to Uses

As indicated in the Summary above, this invention concerns chimeric receptors, which include portions of both metabotropic glutamate receptor and calcium receptor proteins. It also is concerned with fragments of metabotropic glutamate receptors and calcium receptors.

Related aspects include nucleic acids encoding such chimeric receptors and fragments, uses of such receptors, fragments and nucleic acids, and cell lines expressing such nucleic acids. The uses disclosed include methods of screening for compounds that bind to or modulate the activity of metabotropic glutamate receptors or calcium receptors using such chimeric receptors and fragments. The invention also includes compounds for modulating metabotropic glutamate receptors or calcium receptors identified by such methods of screening, and methods for treating certain disorders or for modulating metabotropic glutamate receptors or calcium receptors utilizing such compounds.

Experiments carried out on several distinct G-protein coupled receptors have suggested the general principle that G-protein coupling specificity and receptor desensitization are determined primarily by amino acid sequences which are intracellular (i.e., sequences within one or more of the three cytoplasmic loops and/or the intracellular cytoplasmic tail). Recent experiments in which chimeric receptors were formed by combining distinct protein segments from different metabotropic glutamate receptors (mGlurs), suggest that, in these receptors, ligand binding specificity is determined by the extracellular domain.

Thus, preferred embodiments of the present invention include chimeric receptors consisting of the extracellular domain (ECD) of an mGluR and the seven-transmembrane domain (7TMD) and the intracellular cytoplasmic tail (CT)

of a calcium receptor (CaR) that responds to mGluR-active molecules by signal transduction analogous to that observed when CaR-active molecules act on a CaR.

Similarly, in other preferred embodiments, the invention includes chimeric receptors in which the intracellular cytoplasmic C-terminal tail domain of a chosen mGluR is replaced by the C-terminal tail of a calcium receptor. The C-terminal tail encompasses the cytoplasmic region which follows the seventh transmembrane region.

Preferred embodiments of the invention also include chimeric receptors in which the peptide sequences encompassing all or some of the cytoplasmic loop domains (between the first and second, the third and fourth, and the fifth and sixth transmembrane regions) of an mGluR have been replaced similarly with corresponding peptide sequences from one or more CaRs. In particular such embodiments include chimeric receptors having the ECD of an mGluR, the 7TMD of an mGluR, and the C-terminal tail of a calcium receptor, except that one or more sub-domains of the 7-TMD are substituted with sequences from a CaR. This specifically includes receptors in which one or more of the cytoplasmic loops of the 7TMD are replaced with sequences from a CaR. Such substitution of cytoplasmic loops may be done singly or in any combination. In general, using techniques known to those skilled in the art, such target "domains" and "sub-domains" may be "swapped" individually or in combination.

These chimeric receptors are unknown in the art and their function is unexpected because functional chimeric receptors had previously been successfully constructed only by combining portions of much more closely related receptors. Indeed, the sequence identity between metabotropic glutamate receptors and calcium receptors is only about 19-25%, and the two types of receptors share only about 25-30% sequence similarity (Brown E.M. et al., *Nature* 366:575, 1993).

Experiments have shown that ligands known in the art which are agonists or antagonists on the native mGluRs also exhibit such activities on the chimeric receptors in which the extracellular domain is from an mGluR. Other ligands which bind to the ECD and modulate the activity of mGluRs, for example, agonists, antagonists, allosteric modulators and the like, are also predicted to act on such chimeric receptors. Experiments have also shown that ligands known in the art which modulate mGluRs act on the chimeric receptors in which the ECD and 7TMD are from an mGluR. Other ligands which modulate mGluR activity are also predicted to act on this type of chimeric receptors regardless of whether they bind the ECD or 7TMD of mGluRs.

The chimeric receptors are linked to intracellular or second messenger functions in a similar fashion to the linkage known for non-modified calcium receptors. For example, as is the case for CaRs, the chimeric receptors are also coupled through a G-protein(s) to the activation of phospholipase C, to the generation of inositol phosphates and/or to the release of calcium ions from

intracellular stores. Although the mGluRs rapidly desensitize upon ligand binding/activation, the CaRs do not, allowing for more efficient high-throughput screening of compounds active at the CaR and stable receptor expression in recombinant cell lines. Importantly, the chimeric mGluR/CaR receptors do not rapidly desensitize upon ligand binding/activation and can be therefore efficiently used for high throughput screening. In addition, the chimeric receptors can be functionally expressed in stable cell lines.

Cells expressing such chimeric receptors can be prepared and used in functional assays to identify compounds which modulate activities of selected mGluRs. For example, increases in intracellular calcium levels resulting from receptor activation can be monitored by use of fluorescent calcium chelating dyes. Functional assays have been described for identifying molecules active at calcium receptors (see for example, published PCT patent application "Calcium Receptor-Active Molecules," PCT No. US93/01642 (WO94/18959), published September 1994 hereby incorporated by reference herein in its entirety).

An increasingly common practice in modern drug discovery is the use of various target-site-specific assays to identify specific molecules with activities of interest. These assays select drug lead molecules from large collections or libraries of molecules (e.g., combinatorial libraries, proprietary compound libraries held by large drug companies, etc.). Drug lead molecules are "selected" when they bind to pharmacological targets

of interest and thus potentially modify the activities of these targets. The assays can be of many types including direct binding displacement assays or indirect functional assays. In order to successfully develop and use an assay to isolate lead therapeutic compounds, the target molecule (e.g., receptor) must first be identified and isolated. Many functional assays have been described in the literature for identifying molecules active at various receptors and these provide unique advantages over binding assays. It is not necessary to know, *a priori*, which ligands modulate the activity of the receptor *in vivo*, nor is it necessary to know the exact physiological function of the receptor. Compounds identified in functional assays and in subsequent medicinal chemistry efforts can be used as experimental test compounds to obtain such knowledge.

While eight distinct mGluRs are currently known, their discrete functions remain largely undetailed. Nevertheless, molecules active at mGluRs are sought by pharmaceutical companies because these receptors are found in the central nervous system and are known to be involved in the regulation of processes related to memory, motor functions, pain sensation, neurodegeneration and the like. Thus, compounds which modulate mGluRs may be useful in the treatment of disorders or diseases affecting memory, cognition, and motor function (e.g., in seizures) as well as in the treatment of pain and neurodegenerative disorders (e.g., stroke, Alzheimers disease and the like).

Screens to identify molecules active at mGluRs can be constructed using cloned mGluRs themselves. However, functional screens using native mGluRs are problematic. First, most mGluRs are coupled through G_i proteins and this limits their use in functional assays because G_i proteins are linked to inhibition of adenylate cyclase and changes in adenylate cyclase are not easily measured in high throughput functional screens designed to select drug lead molecules from large compound libraries.

Receptors which couple through other G-proteins to activation of phospholipase C (e.g., G_q-coupled receptors) do not suffer this drawback, so it was initially thought that mGluR1 and mGluR5 could find utility in functional assays because these two mGluRs are coupled through Gq-protein(s) to measurable intracellular functions (e.g., activation of phospholipase C, generation of inositol phosphates and the release of calcium ions from intracellular stores).

A second limitation is presented here, however, because these particular mGluRs rapidly desensitize upon agonist binding. That is, the functional response disappears rapidly and cannot quickly be recovered (see for example Figure 8a). Furthermore, it has not always been possible to obtain fully functional stable cell lines expressing mGluRs regardless of the G-protein to which they couple (Tanabe et al., 1992, *Neuron* 8:169-179; Gabellini et al., 1994, *Neurochem Int.* 24:533-539). Thus, nontrivial technical difficulties must be overcome

in order to use native mGluRs in an optimal manner in high throughput functional screening assays.

The invention described herein overcomes these technical difficulties and provides a much improved screening method by utilizing the more robust aspects of the calcium receptors which do not rapidly desensitize upon ligand binding/activation and can be expressed stably in recombinant vertebrate cells (see for example, Figure 8b and see also published PCT patent application "Calcium Receptor-Active Molecules," PCT No. US93/01642 (WO94/18959), published September 1994, hereby incorporated by reference herein). Thus, for example, by coupling the 7TMD and the CT of the CaR to the extracellular domain of mGluR, or the CT of the CaR to the ECD and 7TMD of the mGluR, the mGluR extracellular domain has the benefit of the Gq coupling property of a CaR, as well as the improved property of a lack of rapid desensitization (see, for example, Figure 8c). Thus, the present invention provides chimeric receptors with ligand binding and activation properties similar to those of the native mGluRs, but with improved second messenger coupling similar to CaRs.

Thus, since the chimeric receptors simplify and enable, efficient, practical and reproducible functional screens to identify mGluR-active molecules, compositions and methods of the present invention are useful for the identification of molecules which modulate mGluR activity or calcium receptor activity. These can, for example, include agonists, antagonists, allosteric modulators, and

the like. For example, chimeric receptors constructed to screen compounds active at metabotropic glutamate receptors may employ the signaling properties of certain domains of a calcium receptor. Such a chimeric receptor would take advantage of certain unique properties associated with the agonist-induced coupling of the calcium receptor to G-proteins which activate phospholipase C and mobilize intracellular calcium. These properties include, for example, the lack of ligand induced down-regulation/desensitization which is associated with ligand activation of metabotropic glutamate receptors. Thus the superior signaling properties of the calcium receptor can be transferred to metabotropic glutamate receptors which normally do not couple to G-proteins that activate phospholipase C and mobilize intracellular calcium such as those which couple to G_i.

In certain embodiments, recombinant cells expressing such chimeric receptors are used in screening methods. The cells will obtain properties, such as those indicated above, which facilitate their use in high-throughput functional assays, and thus provide a more efficient method of screening for compounds which bind to or modulate metabotropic glutamate receptor activity.

Generally, useful chimeric receptors include portions of mGluRs and CaRs, such that the portions confer a desired binding, signal coupling, or other functional characteristic to the chimeric receptor. The length of a sequence from a particular receptor can be of different

sizes in different applications. In addition, the sequence of a portion from a particular receptor may be identical to the corresponding sequence in the mGluR or CaR, or it may be a homologous sequence, which retains the relevant function of the mGluR or CaR sequence. Therefore, chimeric receptors of this invention have an extracellular domain, a seven transmembrane domain, and an intracellular cytoplasmic tail domain. These chimeric receptors have a contiguous sequence of at least 6 amino acids which is homologous to a sequence from an mGluR, and a contiguous sequence of at least 6 amino acids which is homologous to a sequence from a CaR. However, in many cases, the sequences from the mGluR and/or the CaR may be longer than 6 amino acids. Thus, either or both of such sequences may be at least 12, 18, 24, 30, 36, or more amino acids in length.

The portions from the mGluR and the CaR will usually not be the same length. Thus, for example, the sequence from one of those types of receptor may be of a length as indicated above (e.g., et at least 6, 12, 18, 24, 30, 36, or more amino acids), while the rest of the sequence of the chimeric receptor is the same as or homologous to a sequence from the other type of receptor.

In certain embodiments, the portion from at least one receptor type is a subdomain. In this context, "subdomain" refers to a sequence of amino acids which is less than the entire sequence of amino acids for a domain. Examples of subdomains include, but are not limited to, ligand binding domains. Other examples include one of the

cytoplasmic loops or regions of the seven transmembrane domain. Therefore, in certain cases, a chimeric receptor has an extracellular domain, a seven transmembrane domain, and an intracellular cytoplasmic tail domain, which include subdomains. In one example of such chimeric receptors, at least one subdomain is homologous to a subdomain of a calcium receptor and the remaining subdomains and domains are homologous to subdomains and domains of a metabotropic glutamate receptor. In another example, at least one subdomain is homologous to a subdomain of a metabotropic glutamate receptor and the remaining subdomains and domains are homologous to subdomains and domains of a calcium receptor.

In a more specific example, the seven transmembrane domain of a chimeric receptor includes three cytoplasmic loops; at least one cytoplasmic loop is homologous to a cytoplasmic loop of a metabotropic glutamate receptor; or at least one cytoplasmic loop is homologous to a cytoplasmic loop of a calcium receptor. In another specific example, the extracellular domain is homologous to the extracellular domain of a metabotropic glutamate receptor, the seven transmembrane domain is homologous to the seven transmembrane domain of a metabotropic glutamate receptor except that one or more of the cytoplasmic loops of the seven transmembrane domain is homologous to a cytoplasmic loop(s) of a calcium receptor, and the cytoplasmic tail is homologous to the cytoplasmic tail of a calcium receptor. Thus, any of cytoplasmic loops 1, 2, and 3 may be replaced, either singly or in any

combination, with a cytoplasmic loop(s) of a calcium receptor.

In other cases, the chimeric receptor has a domain which has a sequence which is the same as or homologous to the sequence of a domain of an mGluR, or a CaR, or preferably, at least one domain from each of an mGluR and a CaR. More preferably, the chimeric receptor has two domains from one receptor type and one domain from the other receptor type. The compositions of certain preferred embodiments of such chimeric receptors are described below:

A composition comprising a chimeric receptor having:

1. one domain homologous to the extracellular domain of a calcium receptor, one domain homologous to the seven transmembrane domain of a metabotropic glutamate receptor, and one domain homologous to the intracellular cytoplasmic tail domain of a metabotropic glutamate receptor; or

2. one domain homologous to an extracellular domain of a metabotropic glutamate receptor, one domain homologous to the seven transmembrane domain of a calcium receptor, and one domain homologous to the intracellular cytoplasmic tail domain of a calcium receptor; or

3. one domain homologous to an extracellular domain of a metabotropic glutamate receptor, one domain homologous to the seven transmembrane domain of a calcium receptor, and one domain homologous to the

intracellular cytoplasmic tail domain of a metabotropic glutamate receptor; or

4. one domain homologous to the extracellular domain of a calcium receptor, one domain homologous to the seven transmembrane domain of a metabotropic glutamate receptor, and one domain homologous to the intracellular cytoplasmic tail domain of a calcium receptor; or

5. one domain homologous to the extracellular domain of a calcium receptor, one domain homologous to the seven transmembrane domain of a calcium receptor, and one domain homologous to the intracellular cytoplasmic tail domain of a metabotropic glutamate receptor; or

6. one domain homologous to the extracellular domain of a metabotropic glutamate receptor, one domain homologous to the seven transmembrane domain of a metabotropic glutamate receptor, and one domain homologous to the intracellular cytoplasmic tail domain of a calcium receptor; or

7. one domain homologous to the extracellular domain of a metabotropic glutamate receptor, one domain homologous to the seven transmembrane domain of a metabotropic glutamate receptor except that one or more cytoplasmic loops are replaced with a cytoplasmic loop(s) homologous to a cytoplasmic loop(s) of a calcium receptor, and one domain homologous to the intracellular cytoplasmic tail domain of a calcium receptor.

B. Nucleic Acids Encoding Chimeric Receptors

Compositions which include isolated nucleic acid molecules which code for chimeric receptors as described above are also useful in this invention. Such nucleic acid molecules can be isolated, purified, or enriched. Preferably, the nucleic acid is provided as a substantially purified preparation representing at least 75%, more preferably 85%, most preferably 95% of the total nucleic acids present in the preparation.

Such nucleic acid molecules may also be present in a replicable expression vector. The replicable expression vector can be transformed into a suitable host cell to provide a recombinant host cell. Using such transformed host cells, the invention also provides a process for the production of a chimeric receptor, which includes growing, under suitable nutrient conditions, procaryotic or eucaryotic host cells transformed or transfected with a replicable expression vector comprising the nucleic acid molecule in a manner allowing expression of said chimeric receptor.

Uses of nucleic acids encoding chimeric receptors or receptor fragments include one or more of the following: producing receptor proteins which can be used, for example, for structure determination, to assay a molecule's activity on a receptor, to screen for molecules useful as therapeutics and to obtain antibodies binding to the receptor. The chimeras of the present invention are useful for identifying compounds active at either calcium receptors or metabotropic glutamate receptors, or both.

Also, the fragments of the present invention are useful for identifying compounds which bind to or modulate either calcium receptors or metabotropic glutamate receptors, or both.

Thus, the invention also provides, for example, an isolated nucleic acid encoding an extracellular domain of a metabotropic glutamate receptor that is substantially free of the seven transmembrane domain and intracellular cytoplasmic tail domain of that metabotropic glutamate receptor. Similarly, the isolated nucleic acid can encode a metabotropic glutamate receptor that is substantially free of at least one membrane spanning domain portion. In another example, an isolated nucleic acid can encode a metabotropic glutamate receptor that is substantially free of the extracellular domain of that metabotropic glutamate receptor.

C. Metabotropic Glutamate Receptor Fragments and Calcium Receptor Fragments

Receptor fragments are portions of metabotropic glutamate receptors or of calcium receptors. Receptor fragments preferably bind to one or more binding agents which bind to a full-length receptor. Binding agents include ligands, such as glutamate, quisqualate, agonists and antagonists, and antibodies which bind to the receptor. Fragments have different uses such as to select other molecules able to bind to a receptor.

Fragments can be generated using standard techniques such as expression of cloned partial sequences of receptor

DNA and proteolytic cleavage of a receptor protein. Proteins are specifically cleaved by proteolytic enzymes, such as trypsin, chymotrypsin or pepsin. Each of these enzymes is specific for the type of peptide bond it attacks. Trypsin catalyzes the hydrolysis of peptide bonds whose carbonyl group is from a basic amino acid, usually arginine or lysine. Pepsin and chymotrypsin catalyze the hydrolysis of peptide bonds from aromatic amino acids, particularly tryptophan, tyrosine and phenylalanine.

Alternate sets of cleaved protein fragments are generated by preventing cleavage at a site which is susceptible to a proteolytic enzyme. For example, reaction of the ε-amino group of lysine with ethyltrifluorothioacetate in mildly basic solution yields a blocked amino acid residue whose adjacent peptide bond is no longer susceptible to hydrolysis by trypsin. Goldberger *et al.*, *Biochemistry* 1:401, 1962). Treatment of such a polypeptide with trypsin thus cleaves only at the arginyl residues.

Polypeptides also can be modified to create peptide linkages that are susceptible to proteolytic enzyme-catalyzed hydrolysis. For example, alkylation of cysteine residues with β-haloethylamines yields peptide linkages that are hydrolyzed by trypsin. (Lindley, *Nature* 178:647, 1956).

In addition, chemical reagents that cleave polypeptide chains at specific residues can be used. (Witcop, *Adv. Protein Chem.* 16:221, 1961). For example, cyanogen

bromide cleaves polypeptides at methionine residues. (Gross & Witkip, *J. Am. Chem. Soc.* 83: 1510, 1961).

Thus, by treating a metabotropic glutamate receptor, or fragments thereof, with various combinations of modifiers, proteolytic enzymes and/or chemical reagents, numerous discrete overlapping peptides of varying sizes are generated. These peptide fragments can be isolated and purified from such digests by chromatographic methods. Alternatively, fragments can be synthesized using an appropriate solid-state synthetic procedure.

Fragments may be selected to have desirable biological activities. For example, a fragment may include just a ligand binding site. Such fragments are readily identified by those of ordinary skill in the art using routine methods to detect specific binding to the fragment. For example, in the case of a metabotropic glutamate receptor, nucleic acid encoding a receptor fragment can be expressed to produce the polypeptide fragment which is then contacted with a receptor ligand under appropriate association conditions to determine whether the ligand binds to the fragment. Such fragments are useful in screening assays for agonists and antagonists of glutamate, and for therapeutic effects where it is useful to remove glutamate from serum, or other bodily tissues.

Other useful fragments include those having only the external portion, membrane-spanning portion, or intracellular portion of the receptor. These portions are readily identified by comparison of the amino acid

sequence of the receptor with those of known receptors, or by other standard methodology. These fragments are useful for forming chimeric receptors with fragments of other receptors to create a receptor with an intracellular portion which performs a desired function within that cell, and an extracellular portion which causes that cell to respond to the presence of glutamate, or those agonists or antagonists described herein. Chimeric receptor genes when appropriately formulated are useful in genetic therapies for a variety of diseases involving dysfunction of receptors or where modulation of receptor function provides a desirable effect in the patient.

Additionally, chimeric receptors can be constructed such that the intracellular domain is coupled to a desired enzymatic process which can be readily detected by calorimetric, radiometric, luminometric, spectrophotometric or fluorimetric assays and is activated by interaction of the extracellular portion with its native ligand (*e.g.*, glutamate) or agonist and/or antagonists of the invention. Cells expressing such chimeric receptors can be used to facilitate screening of metabotropic glutamate receptor agonists and antagonists, and in some cases inorganic ion receptor agonists and antagonists.

Thus, this invention also provides fragments, or purified polypeptides of calcium receptors, metabotropic glutamate receptors, or chimeric receptors including calcium receptor sequences and metabotropic glutamate receptor sequences. The fragments may be used to screen

for compounds that are active at either metabotropic glutamate or calcium receptors. For example, a fragment including the extracellular domain of a calcium receptor or a metabotropic glutamate receptor may be used in a soluble receptor binding assay to identify which molecules in a combinatorial library can bind the receptor within the region assayed. Such "binding" molecules may be predicted to affect the function of the receptor. Preferred receptor fragments include those having functional receptor activity, a binding site, epitope for antibody recognition (typically at least six amino acids), and/or a site which binds a metabotropic glutamate receptor agonist, antagonist or modulator. Other preferred receptor fragments include those having only an extracellular portion, a transmembrane portion, an intracellular portion, and/or a multiple transmembrane portion (e.g., seven transmembrane portion). Such receptor fragments have various uses such as being used to obtain antibodies to a particular region and being used to form chimeric receptors and fragments of other receptors to create a new receptor having unique properties.

The purified polypeptides or fragments preferably have at least six contiguous amino acids of a metabotropic glutamate receptor or calcium receptor or chimeric receptor. By "purified" in reference to a polypeptide is meant that the polypeptide is in a form (i.e., its association with other molecules) distinct from naturally occurring polypeptide. Preferably, the polypeptide is provided as a substantially purified preparation

representing at least 75%, more preferably 85%, most preferably 95%, of the total protein in the preparation.

In many applications, it is preferable that the purified polypeptide or fragment have more than 6 contiguous amino acids from the metabotropic glutamate receptor or calcium receptor or chimeric receptor. For example, the purified polypeptide can have at least 12, 18, 14, 30, or 36 contiguous amino acids of the "parent" receptor.

Other fragments may be prepared which include only the seven transmembrane domain and the cytoplasmic tail domain of calcium receptors, metabotropic glutamate receptors, or chimeric receptors. Such fragments may be useful, for example, in functional assays to screen for compounds whose site of action is at the seven transmembrane domain.

As indicated above, the invention provides methods of screening for a compound that binds to a receptor, which utilizes receptor fragments. In one example, the method includes the steps of: preparing a nucleic acid sequence encoding a fragment of a receptor; inserting the sequence into a replicable expression vector capable of expressing said fragment in a host cell; transforming a host cell with the vector; recovering the fragment from the host cell; introducing fragment and a test compound into an acceptable medium; and monitoring the binding of the compound to the fragment by physically detectable means. In cases in which the receptor is a metabotropic glutamate receptor, the fragment preferably includes an extracellular domain of the metabotropic glutamate

receptor, or a seven transmembrane domain of the metabotropic glutamate receptor, or a seven transmembrane domain and a cytoplasmic tail domain of a metabotropic glutamate receptor. In cases in which the receptor is a calcium receptor, the fragment preferably includes an extracellular domain of the calcium receptor, a seven transmembrane domain of the calcium receptor, or a seven transmembrane domain and a cytoplasmic tail domain of a calcium receptor.

Certain fragments of metabotropic glutamate receptors and calcium receptors retain the functions of activating one or more of the cellular responses normally activated by the "parent" receptor when contacted with a compound which interacts. Thus, for example, a cellular expressed fragment which includes the 7TMD and CT of an mGluR or a CaR, but do not include the ECD, may activate a cellular response(s) when contacted with a compound which interacts with the 7TMD. Thus, incorporation of such fragments in a cell-based method of screening for compounds which bind to or modulate a metabotropic glutamate receptor or calcium receptor, such as that described herein for chimeric receptors, is useful to identify active compounds which interact with the fragment rather that the deleted sequence.

Isolated fragments of calcium receptors, metabotropic glutamate receptors, or chimeric receptors comprising calcium receptor sequences and metabotropic glutamate receptor sequences may be combined in an *in vitro* functional assay to screen for compounds active at either

receptor. Such an *in vitro* assay, for example, may include a fragment having the extracellular domain of one receptor and a fragment having the seven transmembrane domain and the cytoplasmic tail domain of the other receptor, where the extracellular domain will complement the seven transmembrane/cytoplasmic tail domain fragment *in vitro*. In this way functional chimeric receptors which are useful in a screening assay may be prepared without the need for recombination of the nucleic acids encoding them. Instead, these functional chimeric receptors may be achieved by combining, *in vitro*, portions of different receptors.

Such combinations of fragments provide methods of screening for compounds which bind to or modulate a receptor. An example of such a method includes the steps of: preparing a nucleic acid sequence encoding a first fragment which is a fragment of a first receptor; inserting the sequence into a replicable expression vector capable of expressing that fragment in a host cell; transforming a host cell with the vector; recovering the fragment from the host cell; preparing a nucleic acid sequence encoding a second fragment which is a fragment of a second receptor; inserting the sequence into a replicable expression vector capable of expressing the second fragment in a host cell; transforming a host cell with the vector; recovering the second fragment from the host cell, introducing both the first fragment and the second fragment into an acceptable medium, and monitoring

the binding and modulation of the compound by physically detectable means.

In particular preferred examples, the first fragment includes the extracellular domain of a metabotropic glutamate receptor and the second fragment includes the seven transmembrane domain and the cytoplasmic tail domain of a calcium receptor; the first fragment includes the extracellular domain of a calcium receptor and the second fragment includes the seven transmembrane domain and the cytoplasmic tail domain of a metabotropic glutamate receptor; or the first fragment includes the extracellular domain of a calcium receptor and the second fragment includes the seven transmembrane domain of a metabotropic glutamate receptor and the cytoplasmic tail domain of a calcium receptor.

D. <u>Screening Procedures to Identify Compounds which Modulate Metabotropic Glutamate Receptor Activities Using Chimeric Receptors</u>

The mGluR agonist and antagonist compounds described in the scientific literature are related to the endogenous agonist, glutamate (for reviews see: Cockcroft *et al.*, *Neurochem. Int. 23*:583-594, 1993; Schoepp and Conn, *TIPS 14*:13-20, 1993; Hollmann and Heinemann, *Annu. Rev. Neurosci. 17*:31-108, 1994). Such agonist and antagonist compounds have an acidic moiety, usually a carboxylic acid, but sometimes a phosphatidic acid. Presumably then, such compounds bind mGluRs at the same site as the amino

acid, glutamate. This has been confirmed for methylcarboxyphenylglycine, which was shown to be a competitive antagonist of glutamate (Eaton *et al.*, *Eur. J. Pharm. - Mol. Pharm. Sect. 244*:195-197, 1993). It can be assumed that compounds active at mGluRs, lacking negative charges, and not resembling the amino acid glutamate, may not act at the glutamate binding site.

Compounds targeted to the metabotropic glutamate receptor have several uses including diagnostic uses and therapeutic use. The syntheses of many of the compounds is described by Nemeth *et al.*, entitled "Calcium Receptor Active Molecule" International Publication Number WO 93/04373, hereby incorporated by reference herein. Those compounds binding to a metabotropic glutamate receptor and those compounds efficacious in modulating metabotropic receptor glutamate activity can be identified using the procedures described herein. Those compounds which can selectively bind to the metabotropic glutamate receptor can be used diagnostically to determine the presence of the metabotropic glutamate receptor versus other glutamate receptors.

The following is a description of procedures which can be used to obtain compounds modulating metabotropic glutamate receptor activity. Various screening procedures can be carried out to assess the ability of a compound to modulate activity of chimeric receptors of the invention by measuring its ability to have one or more activities of a metabotropic glutamate receptor modulating agent or a calcium receptor modulating agent. In cells expressing

chimeric receptors of the invention, such activities include the effects on intracellular calcium, inositol phosphates and cyclic AMP.

Measuring $[Ca^{2+}]_i$ with fura-2 provides a very rapid means of screening new organic molecules for activity. In a single afternoon, 10-15 compounds (or molecule types) can be examined and their ability to mobilize or inhibit mobilization of intracellular Ca^{2+} can be assessed by a single experiment. The sensitivity of observed increases in $[Ca^{2+}]_i$ to depression by PMA can also be assessed.

For example, recombinant cells expressing chimeric receptors of the invention loaded with fura-2 are initially suspended in buffer containing 0.5 mM $CaCl_2$. A test substance is added to the cuvette in a small volume (5-15 μl) and changes in the fluorescence signal are measured. Cumulative increases in the concentration of the test substance are made in the cuvette until some predetermined concentration is achieved or no further changes in fluorescence are noted. If no changes in fluorescence are noted, the molecule is considered inactive and no further testing is performed.

In the initial studies, molecules may be tested at concentrations as high as 5 or 10 mM. As more potent molecules became known, the ceiling concentration was lowered. For example, newer molecules are tested at concentrations no greater than 500 μM. If no changes in fluorescence are noted at this concentration, the molecule can be considered inactive.

Molecules causing increases in $[Ca^{2+}]_i$ are subjected to additional testing. Two characteristics of a molecule which can be considered in screening for a positive modulating agent of a chimeric receptor of the invention are the mobilization of intracellular Ca^{2+} and sensitivity to PKC activators.

A single preparation of cells can provide data on $[Ca^{2+}]_i$ cyclic AMP levels, IP and other intracellular messengers. A typical procedure is to load cells with fura-2 and then divide the cell suspension in two; most of the cells are used for measurement of $[Ca^{2+}]_i$ and the remainder are incubated with molecules to assess their effects on cyclic AMP.

Measurements of inositol phosphates are a time-consuming aspect of the screening. However, ion-exchange columns eluted with chloride (rather than formate) provide a very rapid means of screening for IP_3 formation, since rotary evaporation (which takes around 30 hours) is not required. This method allows processing of nearly 100 samples in a single afternoon by a single experimenter. Those molecules that prove interesting, as assessed by measurements of $[Ca^{2+}]_i$, cyclic AMP, and IP_3 can be subjected to a more rigorous analysis by examining formation of various inositol phosphates and assessing their isomeric form by HPLC.

The following is illustrative of methods useful in these screening procedures.

1. <u>Measurement of cyclic AMP</u>

This section describes measuring cyclic AMP levels. Cells were incubated as above and at the end of the incubation, a 0.15-ml sample was taken and transferred to 0.85 ml of hot (70°C) water and heated at this temperature for 5-10 minutes. The tubes were subsequently frozen and thawed several times and the cellular debris sedimented by centrifugation. Portions of the supernatant were acetylated and cyclic AMP concentrations determined by radioimmunoassay.

2. <u>Measurement of Inositol Phosphate Formation</u>

This section describes procedures measuring inositol phosphate formation. Membrane phospholipids were labeled by incubating parathyroid cells with 4 μCi/ml ^{3}H-myo-inositol for 20-24 hours. Cells were then washed and resuspended in PCB containing 0.5 mM $CaCl_2$ and 0.1% BSA. Incubations were performed in microfuge tubes in the absence or presence of various concentrations of organic polycation for different times. Reactions were terminated by the addition of 1 ml chloroform-methanol-12 N HCl (200:100:1; v/v/v). Aqueous phytic acid hydrolysate (200 μl; 25 μg phosphate/tube). The tubes were centrifuged and 600 μl of the aqueous phase was diluted into 10 ml water.

Inositol phosphates were separated by ion-exchange chromatography using AG1-X8 in either the chloride- or formate-form. When only IP_3 levels were to be determined, the chloride-form was used, whereas the formate form was used to resolve the major inositol phosphates (IP_3, IP_2, and IP_1). For determination of just IP_3, the diluted sample was applied to the chloride-form column and the

column was washed with 10 ml 30 mM HCl followed by 6 ml 90 mM HCl and the IP_3 was eluted with 3 ml 500 mM HCl. The last eluate was diluted and counted. For determination of all major inositol phosphates, the diluted sample was applied to the formate-form column and IP_1, IP_2, and IP_3 eluted sequentially by increasing concentrations of formate buffer. The eluted samples from the formate columns were rotary evaporated, the residues brought up in cocktail, and counted.

The isomeric forms of IP_3 were evaluated by HPLC. The reactions were terminated by the addition of 1 ml 0.45 M perchloric acid and stored on ice for 10 minutes. Following centrifugation, the supernatant was adjusted to pH 7-8 with $NaHCO_3$. The extract was then applied to a Partisil SAX anion-exchange column and eluted with a linear gradient of ammonium formate. The various fractions were then desalted with Dowex followed by rotary evaporation prior to liquid scintillation counting in a Packard Tri-carb 1500 LSC.

For all inositol phosphate separation methods, appropriate controls using authentic standards were used to determine if organic polycations interfered with the separation. If so, the samples were treated with cation-exchange resin to remove the offending molecule prior to separation of inositol phosphates.

3. Use of Lead Molecules

By systematically measuring the ability of a lead molecule to mimic or antagonize the effect of a natural ligand, the importance of different functional groups for

agonists and antagonists can be identified. Of the molecules tested, some are suitable as drug candidates while others are not necessarily suitable as drug candidates. The suitability of a molecule as a drug candidate depends on factors such as efficacy and toxicity. Such factors can be evaluated using standard techniques. Thus, lead molecules can be used to demonstrate that the hypothesis underlying receptor-based therapies is correct and to determine the structural features that enable the receptor-modulating agents to act on the receptor and, thereby, to obtain other molecules useful in this invention.

The examples described herein demonstrate the general design of molecules useful as modulators of the activity of mGluRs and CaRs. The examples also describe screening procedures to obtain additional molecules, such as the screening of natural product libraries. Using these procedures, those of ordinary skill in the art can identify other useful modulators of mGluRs and CaRs.

Cell lines expressing calcium receptors have been obtained and methods applicable to their use in high throughput screening to identify compounds which modulate the activity of calcium receptors disclosed (See U.S.S.N. 08/353,784, filed December 9, 1994, hereby incorporated by reference herein). Cell lines expressing metabotropic glutamate receptors have been obtained and methods applicable to their potential use to identify compounds which modulate activity of metabotropic glutamate receptors disclosed (European Patent Publication No. 0 568

384 A1; European Patent Publication No. 0 569 240 A1; PCT Publication No. WO 94/29449; and PCT Publication No. WO 92/10583). Thus, recombinant cell-based assays which use biochemical, spectrophotometric or other physical measurements to detect the modulation of activity of an expressed receptor, especially by measuring changes in affected intracellular messengers, are known to those in the art and can be constructed such that they are suitable for high throughput functional screening of compounds and compound libraries. It will be appreciated by those in the art that each functional assay has advantages and disadvantages for high throughput screening which will vary depending on the receptor of interest, the cell lines employed, the nature of the biochemical and physical measurements used to detect modulation of receptor function, the nature of the compound library being screened and various other parameters. An exceptionally useful and practical method is the use of fluorescent indicators of intracellular Ca^{2+} to detect modulation of the activity of receptors coupled to phospholipase-C.

The use of [^{3}H]glutamate, or any other compound found to modulate the mGluR discovered by the methods described herein, as a lead compound is expected to result in the discovery of other compounds having similar or more potent activity which in turn can be used as lead compounds. Lead compounds such as [^{3}H]glutamate can be used for molecular modeling using standard procedures and to screen compound libraries. Radioligand binding techniques [a radio labeled binding assay] can be used to identify

compounds binding at the glutamate binding site. While such binding assays are useful for finding new compounds binding to the glutamate binding site on mGluR's, the current invention provides for the discovery of novel compounds with unique and useful activities at mGluR's which can be radio labeled and used similarly in Radioligand assays to find additional compounds binding to the new lead defined site. This screening test allows vast numbers of potentially useful compounds to be screened for their ability to bind to the glutamate binding site. Other rapid assays for detection of binding to the glutamate binding site on metabotropic glutamate receptors can be devised using standard detection techniques. Other compounds can be identified which act at the glutamate binding using the procedures described in this section. A high-throughput assay is first used to screen product libraries (*e.g.*, natural product libraries and compound files) to identify compounds with activity at the glutamate (or lead compound) binding site. These compounds are then utilized as chemical lead structures for a drug development program targeting the glutamate or lead compound binding site on metabotropic glutamate receptors. Routine experiments, including animal studies can be performed to identify those compounds having the desired activities.

The following assay can be utilized as a high-throughput assay. Rat brain membranes are prepared according to the method of Williams *et al.* (*Molec. Pharmacol. 36*:575, 1989), with the following alterations:

Male Sprague-Dawley rats (Harlan Laboratories) weighing 100-200 g are sacrificed by decapitation. The cortex or cerebellum from 20 rats are cleaned and dissected. The resulting brain tissue is homogenized at 4°C with a polytron homogenizer at the lowest setting in 300 ml 0.32 M sucrose containing 5 mM K-EDTA (pH 7.0). The homogenate is centrifuged for 10 min at 1,000 x *g* and the supernatant removed and centrifuged at 30,000 x *g* for 30 minutes. The resulting pellet is resuspended in 250 ml 5 mM K-EDTA (pH 7.0) stirred on ice for 15 minutes, and then centrifuged at 30,000 x *g* for 30 minutes. The pellet is resuspended in 300 ml 5 mM K-EDTA (pH 7.0) and incubated at 32°C for 30 minutes. The suspension is then centrifuged at 100,000 x *g* for 30 minutes. Membranes are washed by resuspension in 500 ml 5 mM K-EDTA (pH 7.0), incubated at 32°C for 30 minutes, and centrifuged at 100,000 x *g* for 30 minutes. The wash procedure, including the 30-minute incubation, is repeated. The final pellet is resuspended in 60 ml 5 mM K-EDTA (pH 7.0) and stored in aliquots at -80°C.

To perform a binding assay with [^{3}H]glutamate (as an example of a lead compound), aliquots of SPMs (synaptic plasma membranes) are thawed, resuspended in 30 ml of 30 mM EPPS/1 mM K-EDTA, pH 7.0, and centrifuged at 100,000 x *g* for 30 minutes. SPMs are resuspended in buffer A (30 mM EPPS/1 mM K-EDTA, pH 7.0). The [^{3}H]-glutamate is added to this reaction mixture. Binding assays are carried out in polypropylene test tubes. The final incubation volume is 500 μl. Nonspecific binding is determined in the presence of 100 μM nonradioactive glutamate. Duplicate samples are

incubated at 0°C for 1 hour. Assays are terminated by adding 3 ml of ice-cold buffer A, followed by filtration over glass-fiber filters (Schleicher & Schuell No. 30) that are presoaked in 0.33% polyethyleneimine (PEI). The filters are washed with another 3 x 3 ml of buffer A, and radioactivity is determined by scintillation counting at an efficiency of 35-40% for ^{3}H.

In order to validate the above assay, the following experiments can also be performed:

(a) The amount of nonspecific binding of the [^{3}H]glutamate to the filters is determined by passing 500 μl of buffer A containing various concentrations of [^{3}H]glutamate through the presoaked glass-fiber filters. The filters are washed with another 4 x 3 ml of buffer A, and radioactivity bound to the filters is determined by scintillation counting at an efficiency of 35-40% for ^{3}H.

(b) A saturation curve is constructed by resuspending SPMs in buffer A. The assay buffer (500 μl) contains 60 μg of protein. Concentrations of [^{3}H]glutamate are used, ranging from 1.0 nM to 400 μM in half-log units. A saturation curve is constructed from the data, and an apparent K_D value and B_{max} value determined by Scatchard analysis (Scatchard, *Ann. N.Y. Acad. Sci. 51*: 660, 1949). The cooperativity of binding of the [^{3}H]glutamate is determined by the construction of a Hill plot (Hill, *J. Physiol. 40*:190, 1910).

(c) The dependence of binding on protein (receptor) concentration is determined by resuspending SPMs in buffer A. The assay buffer (500 μl) contains a concentration of

$[^3H]$glutamate equal to its K_D value and increasing concentrations of protein. The specific binding of $[^3H]$glutamate should be linearly related to the amount of protein (receptor) present.

(d) The time-course of ligand-receptor binding is determined by resuspending SPMs in buffer A. The assay buffer (500 μl) contains a concentration of $[^3H]$glutamate equal to its K_D value and 100 μg of protein. Duplicate samples are incubated at 0°C for varying lengths of time; the time at which equilibrium is reached is determined, and this time point is routinely used in all subsequent assays.

(e) The pharmacology of the binding site can be analyzed by competition experiments. In such experiments, the concentration of $[^3H]$glutamate and the amount of protein are kept constant, while the concentration of test (competing) drug is varied. This assay allows for the determination of an IC_{50} and an apparent K_D for the competing drug (Cheng and Prusoff, *J. Biochem. Pharmacol.* *22*:3099, 1973). The cooperativity of binding of the competing drug is determined by Hill plot analysis.

Specific binding of the $[^3H]$glutamate represents binding to the glutamate binding site on metabotropic glutamate receptors. As such, analogs of glutamate should compete with the binding of $[^3H]$glutamate in a competitive fashion, and their potencies in this assay should correlate with their potencies in a functional assay of metabotropic glutamate receptor activity (*e.g.*, electrophysiological assessment of the activity of cloned

metabotropic glutamate receptors expressed in *Xenopus* oocytes). Conversely, compounds which have activity at the sites other that the glutamate binding site should not displace [^{3}H]glutamate binding in a competitive manner. Rather, complex allosteric modulation of [^{3}H]glutamate binding, indicative of noncompetitive interactions, might occur.

(f) Studies estimating the dissociation kinetics are performed by measuring the binding of [^{3}H]glutamate after it is allowed to come to equilibrium (see (d) above), and a large excess of nonradioactive competing drug is added to the reaction mixture. Binding of the [^{3}H]glutamate is then assayed at various time intervals. With this assay, the association and dissociation rates of binding of the [^{3}H]glutamate are determined (Titeler, *Multiple Dopamine Receptors: Receptor Binding Studies in Dopamine Pharmacology*. Marcel Dekker, Inc., New York, 1983). Additional experiments involve varying the reaction temperature (0°C to 37°C) in order to understand the temperature dependence of this parameter.

The following is one example of a rapid screening assay to obtain compounds modulating metabotropic glutamate receptor activity. The screening assay first measures the ability of compounds to bind to recombinant receptors, or receptor fragments containing the glutamate binding site. Compounds binding to the metabotropic glutamate receptor are then tested for their ability to modulate one or more activities at a metabotropic glutamate receptor.

In one procedure, a cDNA or gene clone encoding the chimeric receptor or fragment of a metabotropic glutamate receptor from a suitable organism such as a human is obtained using standard procedures. Distinct fragments of the clone are expressed in an appropriate expression vector to produce the smallest receptor polypeptide(s) obtainable able to bind glutamate. In this way, the polypeptide(s) containing the glutamate binding site is identified. Such experiments can be facilitated by utilizing a stably transfected mammalian cell line (*e.g.*, HEK 293 cells) expressing metabotropic glutamate receptors.

Alternatively, the metabotropic glutamate receptor can be chemically reacted with glutamate chemically modified so that amino acid residues of the metabotropic glutamate receptor which contact (or are adjacent to) the selected compound are modified and thereby identifiable. The fragment(s) of the metabotropic glutamate receptor containing those amino acids which are determined to interact with glutamate and are sufficient for binding to glutamate, can then be recombinantly expressed using standard techniques.

The recombinant polypeptide(s) having the desired binding properties can be bound to a solid-phase support using standard chemical procedures. This solid-phase, or affinity matrix, may then be contacted with glutamate to demonstrate that this compound can bind to the column, and to identify conditions by which the compound may be removed from the solid-phase. This procedure may then be

repeated using a large library of compounds to determine those compounds which are able to bind to the affinity matrix. Bound compounds can then can be released in a manner similar to glutamate. Alternative binding and release conditions may be utilized to obtain compounds capable of binding under conditions distinct from those used for glutamate binding (*e.g.*, conditions which better mimic physiological conditions encountered especially in pathological states). Compounds binding to the glutamate binding site can thus be selected from a very large collection of compounds present in a liquid medium or extract.

In an alternate method, chimeric receptors are bound to a column or other solid phase support. Those compounds which are not competed off by reagents binding to the glutamate binding site on the receptor can then be identified. Such compounds define alternative binding sites on the receptor. Such compounds may be structurally distinct from known compounds and may define chemical classes of agonists or antagonists which may be useful as therapeutics agents.

Modulating metabotropic glutamate receptor activity causes an increase or decrease in a cellular response which occurs upon metabotropic glutamate receptor activation. Cellular responses to metabotropic glutamate receptor activation vary depending upon the type of metabotropic glutamate receptor activated. Generally, metabotropic glutamate receptor activation causes one or more of the following activities: (1) increase in PI

hydrolysis; (2) activation of phospholipase C; (3) increases and decreases in the formation of cyclic adenosine monophosphate (cAMP); (4) decrease in the formation of cAMP; (5) changes in ion channel function; (6) activation of phospholipase D; (7) activation or inhibition of adenylyl cyclase; (8) activation of guanylyl cyclase; (9) increases in the formation of cyclic guanosine monophosphate (cGMP); (10) activation of phospholipase A_2; (11) increases in arachidonic acid release; (12) increases or decreases in the activity of voltage- and ligand- gated ion channels; (13) and increase in intracellular calcium. Inhibition of metabotropic glutamate receptor activation prevents one or more of these activities from occurring.

Activation of a particular metabotropic glutamate receptor refers to an event which subsequently causes the production of one or more activities associated with the type of receptor activated. Activation of mGluR1 can result in one or more of the following activities: increase in PI hydrolysis, increase in cAMP formation, increase in intracellular calcium (Ca^{2+}) and increase in arachidonic acid formation. Compounds can modulate one or more metabotropic glutamate receptor activities by acting as an agonist or antagonist of glutamate binding site activation.

The chimeric receptors of the present invention provide a method of screening for compounds active at mGluRs by the detection of signals produced by CaRs. The chimeric receptors may be used in the screening procedures

described in PCT/US93/01642 (WO94/18959), which are hereby incorporated by reference herein, including methods of screening using fura-2, and measurement of cytosolic Ca^{2+} using cell lines expressing calcium receptors and methods of screening using oocyte expression.

Active compounds identified by the screening methods described herein, may be useful as therapeutic molecules to modulate metabotropic glutamate receptor activity or as a diagnostic agents to diagnose those patients suffering from a disease characterized by an abnormal metabotropic glutamate receptor activity. Preferably the screening methods are used to identify metabotropic glutamate receptor modulators by screening potentially useful molecules for an ability to mimic or block an activity of extracellular glutamate or other metabotropic glutamate receptor agonists on a cell having a metabotropic glutamate receptor and determining whether the molecule has an EC_{50} IC_{50} of less than or equal to 100 μM. More preferably, the molecules tested for its ability to mimic or block an increase in $[Ca^{2+}]_i$ elicited by extracellular glutamate or other mGluR agonists.

Identification of metabotropic glutamate receptor-modulating agents is facilitated by using a high-throughput screening system. High-throughput screening allows a large number of molecules to be tested. For example, a large number of molecules can be tested individually using rapid automated techniques or in combination using a combinatorial library. Individual compounds able to modulate metabotropic glutamate receptor

activity present in a combinatorial library can be obtained by purifying and retesting fractions of the combinatorial library. Thus, thousands to millions of molecules can be screened in a single day. Active molecules can be used as models to design additional molecules having equivalent or increased activity. Preferably the identification method uses a recombinant chimeric metabotropic glutamate receptor. Chimeric receptors can be introduced into different cells using a vector encoding a receptor. Preferably, the activity of molecules in different cells is tested to identify a metabotropic glutamate receptor agonist or metabotropic glutamate receptor antagonist molecule which mimics or blocks one or more activities of glutamate at a first type of metabotropic glutamate receptor but not at a second type of metabotropic glutamate receptor.

As indicated above, the present invention provides a novel method of screening for compounds which modulate metabotropic glutamate receptor activity, by using a chimeric receptor having portions of a metabotropic glutamate receptor and portions of a calcium receptor. In particular receptors of this type, the signaling process of the calcium receptor portion is used to detect modulation of mGluR activity, as various compounds are tested for binding to the mGluR portion. The method of screening can be conducted in a variety of ways, such as utilizing chimeric receptors having different portions from the metabotropic glutamate receptor and calcium receptor. Certain preferred examples are described below.

In one example, the method of screening for a compound that binds to or modulates the activity of a metabotropic glutamate receptor involves preparing a chimeric receptor having an extracellular domain, a seven transmembrane domain, and an intracellular cytoplasmic tail domain. A sequence of at least 6 contiguous amino acids is the same as or homologous to a sequence from a metabotropic glutamate receptor and a sequence of at least 6 contiguous amino acids is the same as or homologous to a sequence from a calcium receptor. The chimeric receptor and a test compound are introduced into a acceptable medium, and the binding of the test compound to the receptor or the modulation of the receptor by the test compound is monitored by physically detectable means in order to identify such binding or modulating compounds. Generally, acceptable media will include those in which a natural ligand of an mGluR and/or a CaR will interact with an mGluR or a CaR.

Often it will be beneficial to use chimeric receptors which have longer sequences from one or both of the mGluR and the CaR. For example, the chimeric receptor can have a sequence of at least 12, 18, 24, 30, 36, or more amino acids the same as or homologous a sequence from one or both of the mGluR or CaR. In one useful chimeric receptor, one domain is homologous to a domain of a metabotropic glutamate receptor and at least one domain is homologous to a domain of a calcium receptor

In a second example, the method of screening for a compound which binds to or modulates the activity of a

metabotropic glutamate receptor utilizes a nucleic acid sequence which encodes a chimeric receptor. The nucleic acid is expressed in a cell, and binding or modulation by a test compound is observed by monitoring the effects of the test compound on the cell. Thus, generally the method includes preparing a nucleic acid sequence encoding a chimeric receptor. The encoded chimeric receptor has an extracellular domain, a seven transmembrane domain, and an intracellular cytoplasmic tail domain. As in the example above, the chimeric receptor has sequences of at least 6 contiguous amino acids which are the same as or homologous to sequences from each of an mGluR and a CaR. Also as indicated above, the sequences from one or both of the mGluR and the CaR may beneficially be longer in particular applications, e.g., at least 12, 18, 24, 30, 36, or more amino acids in length. The nucleic acid sequence is inserted into a replicable expression vector capable of expressing the chimeric receptor in a host cell, and a host cell is transformed with the vector. The transformed host cell and a test compound are introduced into an acceptable medium and the effect of the compound on the host cell is monitored (such as be techniques or assays described above). Preferably, though not necessarily, the host cell is a eukaryotic cell.

The amino acid sequences of the chimeric receptor can be selected in a variety of combinations in particular cases. Thus, a chimeric receptor can include at least one domain which is homologous to a domain of a metabotropic glutamate receptor and at least one domain which is

homologous to a domain of a calcium receptor. A domain(s) of the chimeric receptor can, for example, be homologous the extracellular domain and/or the seven transmembrane domain of a metabotropic glutamate receptor.

Likewise, a chimeric receptor which has three cytoplasmic loops can have at least one loop homologous to a cytoplasmic loop of an mGluR, or at least one loop homologous to a cytoplasmic loop of a CaR, or at least one loop homologous to a cytoplasmic loop of each of the those receptors.

Similarly, in other chimeric receptors, there is a portion of the receptor which is homologous to a sequence of one type of receptor (CaR or mGluR), while the remainder of the chimeric receptor is homologous to the other type of receptor (CaR or mGluR). Thus, the chimeric receptor can have a sequence of at least 6, 12, 18, 24, 30, 36, or more contiguous amino acids which is homologous to a sequence of one of the receptor types with the remainder of the sequence of the chimeric receptor homologous to a sequence from the other receptor type. This further includes cases in which at least one cytoplasmic loop is from one of the receptor types, or at least one domain is from one of the receptor types.

Other combinations of sequences will also be useful in particular applications.

The chimeric metabotropic glutamate/calcium receptors can also be used to screen for compounds active at both metabotropic glutamate receptors and calcium receptors.

This is particularly useful for screening for compounds which interact at different domains or subdomains in an mGluR than in a CaR. Thus, such chimeras are useful for screening for compounds which, for example, act within the extracellular domain of a metabotropic glutamate receptor and also act within the seven transmembrane domain or the cytoplasmic tail domain of a calcium receptor. Such a chimera would include the extracellular domain of a metabotropic glutamate receptor linked to the seven transmembrane domain and cytoplasmic tail of a calcium receptor.

To screen for such compounds, active at both metabotropic glutamate receptors and calcium receptors, compounds would be screened according to the various methods of the present invention, against the chimeric receptor, the calcium receptor, and the metabotropic glutamate receptor. Compounds active at the seven transmembrane domain of the calcium receptor portion of the chimeric receptor should also be active when tested against the calcium receptor itself. A preferred method of screening for such compounds is to first screen them according to the methods of the present invention against a chimeric molecule having the extracellular domain of the metabotropic glutamate receptor, and the seven transmembrane and cytoplasmic tail domains of the calcium receptor and to then screen the positive compounds against both chimeric molecule having the extracellular and seven transmembrane domains of the metabotropic glutamate receptor and the cytoplasmic tail domain of the calcium

receptor, and the calcium receptor itself. Compounds active at both molecules will be positive when tested against all three chimeric receptors.

Conversely, a chimera including the extracellular domain of a calcium receptor linked to the seven transmembrane domain and cytoplasmic tail of a metabotropic glutamate receptor would be useful in screening for compounds that act within the extracellular domain of a calcium receptor and also act within the seven transmembrane domain or the cytoplasmic tail of a metabotropic glutamate receptor. Preferably, the chimeric receptor, which includes the extracellular domain of a calcium receptor and the seven transmembrane domain and the cytoplasmic tail of a metabotropic glutamate receptor, is further modified to include portions of the cytoplasmic tail of a calcium receptor. This more preferred embodiment would thereby obtain the superior signaling properties of the calcium receptor while still being useful for screening for compounds that act at both the calcium receptor and the metabotropic glutamate receptor.

Thus in one aspect the invention features a method of screening for compounds active at both a metabotropic glutamate receptor and a calcium receptor, by preparing a nucleic acid sequence encoding a chimeric receptor. The chimeric receptor has an extracellular domain, a seven transmembrane domain, and an intracellular cytoplasmic tail domain, and at least one domain is homologous to a domain of the metabotropic glutamate receptor and at least one domain is homologous to a domain of a calcium

receptor. The nucleic acid sequence is inserted into a replicable expression vector capable of expressing said chimeric receptor in a host cell, and a host cell is transformed with the vector. The transformed host cell and a test compound are introduced into an acceptable medium, and the effect of the test compound on the cell are monitored.

In general, for each of the above screening methods using chimeric receptors, the portion of the chimeric receptor homologous to an mGluR and the portion homologous to a CaR are selected to provide the binding, modulation, and/or signal coupling characteristics appropriate for a particular application.

E. Site of Action

The chimeric receptor molecules are also useful in methods for determining the site-of-action of compounds already identified as metabotropic glutamate receptor or calcium receptor active compounds. For example, chimeras including the extracellular domain of a metabotropic glutamate receptor linked to the seven transmembrane domain and cytoplasmic tail of a calcium receptor, as well as chimeras including the extracellular domain of a calcium receptor linked to the seven transmembrane domain and cytoplasmic tail of a metabotropic glutamate receptor would be useful in determining the site-of-action of either metabotropic glutamate receptor or calcium receptor active compounds. Those of ordinary skill in the art will recognize that these are two examples of large sequence

exchanges and that much smaller sequence exchanges may also be employed to further refine the determination of the site-of-action.

Thus, the invention provides a method of determining the site-of-action of a metabotropic glutamate receptor active compound by: preparing a nucleic acid sequence encoding a chimeric receptor wherein the chimeric receptor comprises at least a 6 amino acid sequence which is homologous to a sequence of amino acids of a calcium receptor and the remainder of the amino acid sequence is homologous to a sequence of amino acids of a metabotropic glutamate receptor; inserting the sequence into a replicable expression vector capable of expressing the chimeric receptor in a host cell; transforming a host cell with the vector; introducing the transformed host cell and the compound into an acceptable medium; and monitoring the effect of the compound on the cell.

As indicated above for methods of screening, in particular applications it is advantageous to use sequence exchanges of different sizes. Thus, in other applications, the sequence homologous to a sequence from a calcium receptor, may for example, be at least 12, 18, 24, 30, 36, or more amino acids in length.

Conversely, a method of determining the site-of-action of a calcium receptor active compound can be performed in the same manner as described above, but using a nucleic acid encoding a chimeric receptor which includes at least a 6 amino acid sequence which is homologous to a sequence of amino acids of a metabotropic glutamate receptor and

the remainder of the amino acid sequence is homologous to a sequence of amino acids of a calcium receptor. Also similar to the method above, the sequence homologous to a sequence from a metabotropic glutamate receptor can be of different lengths in various applications, for example, at least 12, 18, 24, 30, 36, or more amino acids in length.

F. Modulation of Metabotropic Glutamate Receptor Activity

Modulation of metabotropic glutamate receptor activity can be used to produce different effects such as anticonvulsant effects, neuroprotectant effects, analgesic effects, cognition-enhancement effects, and muscle-relaxation effects. Each of these effects has therapeutic applications. Compounds used therapeutically should have minimal side effects at therapeutically effective doses.

The ability of a compound to modulate metabotropic glutamate activity can be determined using electrophysiological and biochemical assays measuring one or more metabotropic glutamate activities. In general, such assays can be carried out using cells expressing the metabotropic glutamate receptor(s) of interest, but the assays can also be carried out using cells expressing a chimeric receptors of this invention which modulates the cellular activity which is to be monitored. Examples of such assays include the electrophysiological assessment of metabotropic glutamate receptor function in *Xenopus* oocytes expressing cloned metabotropic glutamate receptors, the electrophysiological assessment of

metabotropic glutamate receptor function in transfected cell lines (*e.g.*, CHO cells, HEK 293 cells, *etc.*) expressing cloned metabotropic glutamate receptors, the biochemical assessment of PI hydrolysis and cAMP accumulation in transfected cell lines expressing cloned metabotropic glutamate receptors, the biochemical assessment of PI hydrolysis and cAMP accumulation in rat brain (*e.g.*, hippocampal, cortical, striatal, *etc.*) slices, fluorimetric measurements of cytosolic Ca^{2+} in cultured rat cerebellar granule cells, and fluorimetric measurements of cytosolic Ca^{2+} in transfected cell lines expressing cloned metabotropic glutamate receptors.

Prior to therapeutic use in a human, the compounds are preferably tested *in vivo* using animal models. Animal studies to evaluate a compound's effectiveness to treat different diseases or disorders, or exert an effect such as an analgesic effect, a cognition-enhancement effect, or a muscle-relaxation effect, can be carried out using standard techniques.

G. Novel Agents and Pharmaceutical Compositions

The chimeric receptors and screening methods described herein provide metabotropic glutamate receptor-binding agents (*e.g.*, compounds and pharmaceutical compositions) discovered due to their ability to bind to a chimeric metabotropic glutamate receptor. Such binding agents are preferably modulators of a metabotropic glutamate receptor. Certain of these agent will be novel compounds

identified by the screening methods described herein. In addition, other such compounds are derived by standard methodology from such identified compounds when such identified compounds are used as lead compounds in screening assays based on analogs of identified active compounds, or in medicinal chemistry developments using identified compounds as lead compounds.

Further, by providing novel and efficient screening methods using chimeric receptors, this invention provides a method for preparing a pharmaceutical agent active on a metabotropic glutamate receptor. Without such this efficient method, such agents would not be identified. The method involves identifying a active agent by screening using a chimeric receptor of the type described herein in a screening method as described above. The identified agent or an analog of that agent is synthesized in an amount sufficient to administer to a patient in a therapeutically effective amount.

H. Treatment of Diseases and Disorders

A preferred use of the compounds and methods of the present invention is in the treatment of neurological diseases and disorders. Patients suffering from a neurological disease or disorder can be diagnosed by standard clinical methodology.

Neurological diseases or disorders include neuronal degenerative diseases, glutamate excitotoxicity, global and focal ischemic and hemorrhagic stroke, head trauma,

spinal cord injury, hypoxia-induced nerve cell damage, and epilepsy. These different diseases or disorders can be further medically characterized. For example, neuronal degenerative diseases include Alzheimer's disease and Parkinson's disease.

Another preferred use of the present invention is in the production of other therapeutic effects, such as analgesic effects, cognition-enhancement effects, or muscle-relaxation effects. The present invention is preferably used to produce one or more of these effects in a patient in need of such treatment.

Patients in need of such treatment can be identified by standard medical techniques. For example, the production of analgesic activity can be used to treat patients suffering from clinical conditions of acute and chronic pain including the following: preemptive preoperative analgesia; peripheral neuropathies such as occur with diabetes mellitus and multiple sclerosis; phantom limb pain; causalgia; neuralgias such as occur with herpes zoster; central pain such as that seen with spinal cord lesions; hyperalgesia; and allodynia.

In a method of treating a patient, a therapeutically effective amount of a compound which *in vitro* modulates the activity of a chimeric receptor having at least the extracellular domain of a metabotropic glutamate receptor is administered to the patient. Typically, the compound modulates metabotropic glutamate receptor activity by acting as an agonist or antagonist of glutamate binding site activation. Preferably, the patient has a

neurological disease or a disorder, preferably the compound has an effect on a physiological activity. Such physiological activity can be convulsions, neuroprotection, neuronal death, neuronal development, central control of cardiac activity, waking, control of movements and control of vestibo ocular reflex.

Diseases or disorders which can be treated by modulating metabotropic glutamate receptor activity include one or more of the following types: (1) those characterized by abnormal glutamate homeostasis; (2) those characterized by an abnormal amount of an extracellular or intracellular messenger whose production can be affected by metabotropic glutamate receptor activity; (3) those characterized by an abnormal effect (*e.g.*, a different effect in kind or magnitude) of an intracellular or extracellular messenger which can itself be ameliorated by metabotropic glutamate receptor activity; and (4) other diseases or disorders in which modulation of metabotropic glutamate receptor activity will exert a beneficial effect, for example, in diseases or disorders where the production of an intracellular or extracellular messenger stimulated by receptor activity compensates for an abnormal amount of a different messenger.

The compounds and methods can also be used to produce other effects such as an analgesic effect, cognition-enhancement effect, and a muscle-relaxant effect.

A "patient" refers to a mammal in which modulation of an metabotropic glutamate receptor will have a beneficial effect. Patients in need of treatment involving

modulation of metabotropic glutamate receptors can be identified using standard techniques known to those in the medical profession. Preferably, a patient is a human having a disease or disorder characterized by one more of the following: (1) abnormal glutamate receptor activity (2) an abnormal level of a messenger whose production or secretion is affected by metabotropic glutamate receptor activity; and (3) an abnormal level or activity of a messenger whose function is affected by metabotropic glutamate receptor activity.

By "therapeutically effective amount" is meant an amount of an agent which relieves to some extent one or more symptoms of the disease or disorder in the patient; or returns to normal either partially or completely one or more physiological or biochemical parameters associated with or causative of the disease.

More generally, this invention provides a method for modulating metabotropic glutamate receptor activity by providing to a cell having a metabotropic glutamate receptor an amount of a metabotropic glutamate receptor-modulating molecule sufficient to either mimic one or more effects of glutamate at the metabotropic glutamate receptor, or block one or more effects of glutamate at the metabotropic glutamate receptor. The method can carried out *in vitro* or *in vivo*.

I. Formulation and Administration

Active compounds as identified by the methods of this invention can be utilized as pharmaceutical agents or

compositions to treat different diseases and disorders as described above. In this context, a pharmacological agent or composition refers to an agent or composition in a form suitable for administration to a mammal, preferably a human.

The optimal formulation and mode of administration of compounds of the present invention to a patient depend on factors known in the art such as the particular disease or disorder, the desired effect, and the type of patient. While the compounds will typically be used to treat human patients, they may also be used to treat similar or identical diseases in other vertebrates such as other primates, farm animals such as swine, cattle and poultry, and sports animals and pets such as horses, dogs and cats.

Preferably, the therapeutically effective amount is provided as a pharmaceutical composition. A pharmacological agent or composition refers to an agent or composition in a form suitable for administration into a multicellular organism such as a human. Suitable forms, in part, depend upon the use or the route of entry, for example oral, transdermal, or by injection. Such forms should allow the agent or composition to reach a target cell whether the target cell is present in a multicellular host or in culture. For example, pharmacological agents or compositions injected into the blood stream should be soluble. Other factors are known in the art, and include considerations such as toxicity and forms which prevent the agent or composition from exerting its effect.

The claimed compositions can also be formulated as pharmaceutically acceptable salts (*e.g.*, acid addition salts) and/or complexes thereof. Pharmaceutically acceptable salts are non-toxic salts at the concentration at which they are administered. The preparation of such salts can facilitate the pharmacological use by altering the physical-chemical characteristics of the composition without preventing the composition from exerting its physiological effect. Examples of useful alterations in physical properties include lowering the melting point to facilitate transmucosal administration and increasing the solubility to facilitate the administration of higher concentrations of the drug.

Pharmaceutically acceptable salts include acid addition salts such as those containing sulfate, hydrochloride, phosphate, sulfamate, acetate, citrate, lactate, tartrate, methanesulfonate, ethanesulfonate, benzenesulfonate, *p*-toluenesulfonate, cyclohexylsulfamate and quinate. (*See e.g., supra.* PCT/US92/03736.) Pharmaceutically acceptable salts can be obtained from acids such as hydrochloric acid, sulfuric acid, phosphoric acid, sulfamic acid, acetic acid, citric acid, lactic acid, tartaric acid, malonic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, *p*-toluenesulfonic acid, cyclohexylsulfamic acid, and quinic acid.

Pharmaceutically acceptable salts can be prepared by standard techniques. For example, the free base form of a compound is dissolved in a suitable solvent, such as an aqueous or aqueous-alcohol solution, containing the

appropriate acid and then isolated by evaporating the solution. In another example, a salt is prepared by reacting the free base and acid in an organic solvent.

Carriers or excipients can also be used to facilitate administration of the compound. Examples of carriers and excipients include calcium carbonate, calcium phosphate, various sugars such as lactose, glucose, or sucrose, or types of starch, cellulose derivatives, gelatin, vegetable oils, polyethylene glycols and physiologically compatible solvents. The compositions or pharmaceutical composition can be administered by different routes including intravenously, intraperitoneal, subcutaneous, and intramuscular, orally, topically, or transmucosally.

The compounds of the invention can be formulated for a variety of modes of administration, including systemic and topical or localized administration. Techniques and formulations generally may be found in *Remington's Pharmaceutical Sciences*, 18th Edition, Mack Publishing Co., Easton, PA, 1990.

For systemic administration, oral administration is preferred. For oral administration, the compounds are formulated into conventional oral dosage forms such as capsules, tablets and tonics.

Alternatively, injection may be used, *e.g.*, intramuscular, intravenous, intraperitoneal, subcutaneous, intrathecal, or intracerebroventricular. For injection, the compounds of the invention are formulated in liquid solutions, preferably in physiologically compatible buffers such as Hank's solution or Ringer's solution.

Alternatively, the compounds of the invention are formulated in one or more excipients (*e.g.*, propylene glycol) that are generally accepted as safe as defined by USP standards. In addition, the compounds may be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms are also included.

Systemic administration can also be by transmucosal or transdermal means, or the molecules can be administered orally. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, bile salts and fusidic acid derivatives. In addition, detergents may be used to facilitate permeation. Transmucosal administration may be, for example, through nasal sprays or using suppositories. For oral administration, the molecules are formulated into conventional oral administration dosage forms such as capsules, tablets, and liquid preparations.

For topical administration, the compounds of the invention are formulated into ointments, salves, gels, or creams, as is generally known in the art.

The amounts of various compounds to be administered can be determined by standard procedures. Generally, a therapeutically effective amount is between about 1 nmole and 3 μmole of the molecule, preferably 0.1 nmole and 1 μmole depending on its EC_{50} or IC_{50} and on the age and size of the patient, and the disease or disorder associated

with the patient. Generally, it is an amount between about 0.1 and 50 mg/kg, preferably 0.01 and 20 mg/kg of the animal to be treated.

J. Transgenic Animals

The invention also provides transgenic, nonhuman mammals containing a transgene encoding a chimeric receptor, particularly a chimeric metabotropic glutamate receptor. Transgenic nonhuman mammals are particularly useful as an *in vivo* test system for studying the effects of introducing a chimeric receptor. Experimental model systems may be used to study the effects in cell or tissue cultures, in whole animals, or in particular cells or tissues within whole animals or tissue culture systems. The effects can be studied over specified time intervals (including during embryogenesis).

The present invention provides for experimental model systems for studying the physiological effects of the receptors. Model systems can be created having varying degrees of receptor expression. For example, the nucleic acid encoding a receptor may be inserted into cells which naturally express the parent receptors, such that the chimeric gene is expressed at much higher levels. Also, a recombinant gene may be used to inactivate the endogenous gene by homologous recombination, and thereby create a receptor deficient cell, tissue, or animal.

Inactivation of a gene can be caused, for example, by using a recombinant gene engineered to contain an insertional mutation (*e.g.*, the *neo* gene). The

recombinant gene is inserted into the genome of a recipient cell, tissue or animal, and inactivates transcription of the receptor. Such a construct may be introduced into a cell, such as an embryonic stem cell, by techniques such as transfection, transduction, and injection. Stem cells lacking an intact receptor sequence may generate transgenic animals deficient in the receptor.

Preferred test models are transgenic animals. A transgenic animal has cells containing DNA which has been artificially inserted into a cell and inserted into the genome of the animal which develops from that cell. Preferred transgenic animals are primates, mice, rats, cows, pigs, horses, goats, sheep, dogs and cats.

A variety of methods are available for producing transgenic animals. For example, DNA can be injected into the pronucleus of a fertilized egg before fusion of the male and female pronuclei, or injected into the nucleus of an embryonic cell (*e.g.*, the nucleus of a two-cell embryo) following the initiation of cell division (Brinster *et al.*, *Proc. Nat. Acad. Sci. USA* 82: 4438-4442, 1985)). By way of another example, embryos can be infected with viruses, especially retroviruses, modified to carry chimeric receptor nucleotide sequences of the present invention.

Pluripotent stem cells derived from the inner cell mass of the embryo and stabilized in culture can be manipulated in culture to incorporate nucleotide sequences of the invention. A transgenic animal can be produced from such stem cells through implantation into a

blastocyst that is implanted into a foster mother and allowed to come to term. Animals suitable for transgenic experiments can be obtained from standard commercial sources such as Charles River (Wilmington, MA), Taconic (Germantown, NY), and Harlan Sprague Dawley (Indianapolis, IN).

Methods for the culturing of embryonic stem (ES) cells and the subsequent production of transgenic animals by the introduction of DNA into ES cells using methods such as electroporation, calcium phosphate/DNA precipitation and direct injection also are well known to those of ordinary skill in the art. See, for example, *Teratocarcinomas and Embryonic Stem Cells, A Practical Approach*, E.J. Robertson, ed., IRL Press (1987).

Procedures for embryo manipulations are well known in the art. The procedures for manipulation of the rodent embryo and for microinjection of DNA into the pronucleus of the zygote are well known to those of ordinary skill in the art (Hogan *et al.*, *supra*). Microinjection procedures for fish, amphibian eggs and birds are detailed in Houdebine and Chourrout (*Experientia* 47:897-905, 1991). Other procedures for introduction of DNA into tissues of animals are described in U.S. Patent No. 4,945,050 (Sandford *et al.*, July 30, 1990).

Transfection and isolation of desired clones can be carried out using standard techniques (*e.g.*, E.J. Robertson, *supra*). For example, random gene integration can be carried out by co-transfecting the nucleic acid with a gene encoding antibiotic resistance.

Alternatively, for example, the gene encoding antibiotic resistance is physically linked to a nucleic acid sequence encoding a chimeric receptor of the present invention.

DNA molecules introduced into ES cells can also be integrated into the chromosome through the process of homologous recombination. (Capecchi, *Science* 244: 1288-1292, 1989). Methods for positive selection of the recombination event (*e.g.*, neomycin resistance) and dual positive-negative selection (*e.g.*, neomycin resistance and gancyclovir resistance) and the subsequent identification of the desired clones by PCR have been described by Capecchi, *supra* and Joyner *et al.*, *Nature* 338:153-156, 1989), the teachings of which are incorporated herein.

The final phase of the procedure is to inject targeted ES cells into blastocysts and to transfer the blastocysts into pseudopregnant females. The resulting chimeric animals are bred and the offspring are analyzed by Southern blotting to identify individuals that carry the transgene.

An example describing the preparation of a transgenic mouse is as follows. Female mice are induced to superovulate and placed with males. The mated females are sacrificed by CO_2 asphyxiation or cervical dislocation and embryos are recovered from excised oviducts. Surrounding cumulus cells are removed. Pronuclear embryos are then washed and stored until the time of injection.

Randomly cycling adult female mice paired with vasectomized males serve as recipients for implanted embryos. Recipient females are mated at the same time as

donor females and embryos are transferred surgically to recipient females.

The procedure for generating transgenic rats is similar to that of mice. See Hammer *et al.*, *Cell* 63:1099-1112, 1990). Procedures for the production of transgenic non-rodent mammals and other animals are known in art. See, for example, Houdebine and Chourrout, *supra*; Pursel *et al.*, *Science* 244:1281-1288, 1989); and Simms *et al.*, *Bio/Technology* 6:179-183, 1988).

K. Transfected Cell Lines

Nucleic acid expressing a functional chimeric receptor can be used to create transfected cell lines which functionally express a specific chimeric receptor. Such cell lines have a variety of uses such as being used for high-throughput screening for molecules able to modulate metabotropic glutamate receptor activity; and being used to assay binding to a metabotropic glutamate receptor.

A variety of cell lines are capable of coupling exogenously expressed receptors to endogenous functional responses. A number of these cell lines (*e.g.*, NIH-3T3, HeLa, NG115, CHO, HEK 293 and COS7) can be tested to confirm that they lack an endogenous metabotropic glutamate. Those lines lacking a response to external glutamate can be used to establish stably transfected cell lines expressing the cloned chimeric receptors of the invention.

Production of these stable transfectants is accomplished by transfection of an appropriate cell line

with a eukaryotic expression vector, such as pMSG, in which the coding sequence for the chimeric metabotropic glutamate receptor cDNA has been cloned into the multiple cloning site. These expression vectors contain a promoter region, such as the mouse mammary tumor virus promoter (MMTV), that drive high-level transcription of cDNAS in a variety of mammalian cells. In addition, these vectors contain genes for the selection of cells that stably express the cDNA of interest. The selectable marker in the PMSG vector encodes an enzyme, xanthine-guanine phosphoribosyl transferase (XGPRT), that confers resistance to a metabolic inhibitor that is added to the culture to kill the nontransfected cells. A variety of expression vectors and selection schemes are usually assessed to determine the optimal conditions for the production of metabotropic glutamate receptor-expressing cell lines for use in high-throughput screening assays.

The most effective method for transfection of eukaryotic cell lines with plasmid DNA varies with the given cell type. The chimeric receptor expression construct will be introduced into cultured cells by the appropriate technique, either Ca^{2+} phosphate precipitation, DEAE-dextran transfection, lipofection or electroporation.

Cells that have stably incorporated or are episomally maintaining the transfected DNA will be identified by their resistance to selection media, as described above, and clonal cell lines will be produced by expansion of resistant colonies. The expression of the chimeric metabotropic glutamate receptor cDNA by these cell lines

will be assessed by solution hybridization and Northern blot analysis. Functional expression of the receptor protein will be determined by measuring the mobilization of intracellular Ca^{2+} in response to externally applied calcium receptor agonists.

The following examples illustrate the invention, but do not limit its scope.

III. Examples

Examples are provided below to illustrate different aspects and embodiments of the present invention. These examples are not intended in any way to limit the disclosed invention. Rather, they illustrate methodologies by which the novel chimeric receptors of the present invention may be constructed. They also illustrate methodologies by which compounds may be screened to determine which compounds bind to or modulate a desired mGluR.

Example 1: phPCaR4.0 and pmGluR1s

Plasmid phPCaR4.0 (Garrett et al., J. Biol. Chem., 270:12919, 1995, hereby incorporated by reference herein) was isolated from E. coli bacterial cells containing the plasmid grown up in nutrient broth containing 100 ug/ml ampicillin (Boerhringer Mannheim). This plasmid DNA was used as the source for the DNA encoding the human calcium receptor which was cloned into the EcoRI site of vector pBluescript SK (Stratagene) in the T7 orientation. All

restriction enzymes and modification enzymes were purchased from New England Biolabs unless otherwise noted.

Plasmid p7-3/6A was assembled in pBluescript SK from two overlapping subclones of rat mGluR1 obtained from an oligonucleotide screen of a commercially available rat olfactory bulb cDNA library (Stratagene). This plasmid DNA was used as the source of the metabotropic glutamate receptor, mGluR1. It was also used to screen a commercially available human cerebellar cDNA library for the human analogue. The human cerebellar library was screened with a radioactively labeled rat mGluR1 by a method described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Chapter 1, 1989. Positive plaques were rescued using the manufacturer's protocol and restriction mapped to compare them against the published human mGluR1 sequence (Eur. Patent publications 0 569 240 A1 and 0 568 384 A1). Two subclones were assembled to create a complete human mGluR1.

Alternatively, the sequence of human mGluR1 may be obtained from European Publication Nos. 0 569 240 A1 and 0 568 384 A1. Probes prepared using this sequence may be used to probe human cDNA libraries to obtain the full length human clone. In addition, the relevant sequences may be synthesized using the sequence described therein.

<u>Example 2: pmGluR1/CaR</u>

Chimeric receptors were constructed using recombinant PCR and a multi-step cloning strategy. An overview of recombinant PCR is presented by R. Higuchi in *PCR*

Protocols: A Guide to Methods and Applications, (1990) Academic Press, Inc. In the first construct recombinant PCR was used to combine the sequences of mGluR1 and the CaR across the junction of the extracellular and transmembrane domains. The first chimera, pR1/CaR. contained the extracellular domain of mGluR1 and the transmembrane and intracellular region of the calcium receptor. The chimeric junction was created using three separate PCR reactions. The first reaction used two primers specific for rat mGluR1, A4, a 22 mer encoding nucleotides 1146 to 1167, and an antisense primer, oligoB, a 43 mer containing 22 bases of mGluR1 (nucleotides -1755 to -1776) and 21 bases from the CaR (nucleotides -1837 to -1857). These primers were used to amplify a 650 bp fragment of rat mGluR1. In a separate PCR reaction, a 500 bp fragment of the CaR was amplified using hybrid primer C, a 43 mer which was the complement of oligo B, and D4, an antisense primer corresponding to nucleotides-2256 to -2279 of the CaR. These two PCR products were purified from an agarose gel and annealed together in equal molar ratio in the presence of the external primers A4 and D4 and the proof-reading DNA polymerase, Pfu (Stratagene). The 1,100 bp chimeric PCR product was digested with Nsi I and subcloned into phCar4.0 digested with EcoRV and Nsi I. The resultant subclone was subsequently digested with Xho I and Sfi I to remove the extracellular domain of the CaR which was then replaced with the Xho I- Sfi I fragment of rat mGluR1. The resultant chimera, pR1/Car was validated by

restriction mapping and double-stranded DNA sequencing with Sequenase Version 2.0 (US Biochemical). The DNA sequence for pR1/Car and the corresponding amino acid sequence is depicted in Figure 2.

Example 3: pCaR/R1

A second construct, pCaR/R1, was a reciprocal of the chimera described in example 2 in that it encoded the extracellular domain of CaR and the transmembrane and intracellular region of mGluR1. The chimeric junction was created as described above using recombinant PCR. The first reaction used two primers specific for CaR, CRSf1, a 22 mer corresponding to nucleotides 862 to 883 , and an antisense primer, CR1794, a 36 mer with 18 bases corresponding to CaR (nucleotides -1777 to -1794) and 18 bases from mGluR1(nucleotides -2110 to -2127). These primers were used to amplify a 935 bp fragment of CaR. In a separate PCR reaction, a 360 bp fragment of mGluR1 was amplified using hybrid primer R12110, a 36 mer containing 18 bases of CaR (nucleotides 1777 to 1794) covalently attached to 18 bases of mGluR1 (nucleotides 2110 to 2127) and R1Bgl, an antisense primer corresponding to nucleotides -2451 to -2470 of mGluR1. These two PCR products were purified from an agarose gel and annealed together in equal molar ratio in the presence of the external primers CRSf1 and R1Bgl and the proof-reading DNA polymerase, Pfu (Stratagene). The 1,250 bp chimeric PCR product was digested with Sfi I and Bgl II and subcloned into p7/3A digested with the same enzymes. A subclone

was subsequently digested with Sal I and SfiI to remove the extracellular domain of mGluR1 which was then replaced with the Sal I-Sfi I fragment of CaR. The resultant chimera, pCaR/R1 was validated by restriction mapping and double-stranded DNA sequencing using Sequenase Version 2.0 (US Biochemical). The DNA sequence is for pCaR/R1 and the corresponding amino acid sequence is depicted in Figure 3.

Example 4: pratCH3 and phCH4

These chimeras are a result of swapping the CaR cytoplasmic tail onto the extracellular and transmembrane domains of either rat or human mGluR1. Recombinant PCR was used to attach the C-terminal tail of the CaR onto human mGluR1 (which encodes the rat mGluR1 signal sequence) after nucleotide 2535. The first PCR reaction used two primers specific for human mGluR1, M-1rev a 24 mer corresponding to nucleotides 2242 to 2265 , and an antisense primer, CH3R1, a 36 mer composed of 18 bases of hmGluR1 (nucleotides -2518 to -2535) and 18 bases of CaR (nucleotides -2602 to -2619). These primers were used to amplify a 300 bp fragment of hmGluR1. In a separate PCR reaction, a 750 bp fragment of the CaR was amplified using hybrid primer CH3CaR, a 36 mer which is the complement of oligo CH3R1, and a commercially available T3 primer (Stratagene) which primes in the Bluescript vector in a region downstream from the 3′ end of the CaR. The two PCR products were purified from an agarose gel and annealed together in equal molar ratio in the presence of the external primers M-1 rev and T3 and the proof-reading DNA

polymerase, Pfu (Stratagene). The 1kb chimeric PCR product was digested with Nhe I and Not I and subcloned into phmGluR1 digested with the same enzymes. The resultant chimera, phCH4 was validated by restriction mapping and double-stranded DNA sequencing. To detect functional activity in the oocyte assay with this clone it was necessary to exchange the 5' untranslated region and the signal sequence from rat mGluR1 with the same region of this human clone. This was done utilizing a Bsu36I restriction site. Additionally, an Acc I fragment of rat mGluR1 was subcloned into phCH4 to create a rat version of this same chimera. This chimera is referred to as ratCH3. The DNA sequence for pratCh3 and the corresponding amino acid sequence are depicted in Figure 4. The DNA sequence for phCH44 and the corresponding amino acid sequence are depicted in Figure 5.

Using the techniques described in the above-mentioned examples, we therefore envision the construction, evaluation and screening utility of other mGluR/CaR chimeras. In this example we have taken a Group I metabotropic glutamate receptor which, similar to the calcium receptor, is coupled to the activation of phospholipase C and mobilization of intracellular calcium, and by swapping the C-terminal tail, maintained the integrity of the second messenger system. Additionally, when the CaR tail was added to mGluR1, the desensitization properties were lost. This demonstrates the feasibility of changing specific G-protein coupling of metabotropic glutamate receptors to those of the CaR by swapping

intracellular domains. By example, Group II mGluRs, such as mGluR2 or mGluR3 which are G_i coupled, could be changed to Gq coupled receptors. This can be done by exchanging onto these receptors the C-terminal cytosolic tail of the CaR using the protocol described in examples 2, 3 and 4. Effective Gq coupling could be evaluated in the oocyte as described in examples 5 and 6. Activation of a Group II by L-CCG-I (their most potent agonist), should induce mobilization of intracellular Ca2+ which will cause the detectable inward rectifying C1- current measured in the voltage-clamped oocyte.

To increase the effectiveness of G-protein binding it may be useful to swap one or more additional intracellular (cytoplasmic) loops of the CaR onto the mGluR1. By example, such substitution can involve any of: intracellular loop 1, intracellular loop 2 and intracellular loop 3 from a calcium receptor, substituted alone or in any combination of loops. Such subdomain swapping may be necessary for the most effective transference of G-protein binding specificity.

Example 5: *In vitro* transcription of RNA

RNA transcripts encoding the receptors described in examples 1 through 4 were produced by enzymatic transcription from plasmid templates using T7 polymerase supplied with the mMessage mMachine ™(Ambion). Each plasmid was treated with a restriction enzyme to make a single cut distal to the 3′ end of the cDNA insert to linearize the template. This DNA was incubated with T7

RNA polymerase in the presence of GpppG cap nucleotide, rATP, rCTP, rUTP and rGTP. The synthetic RNA transcript is purified by DNase treatment of the reaction mix and subsequent alcohol precipitations. RNA was quantitated by absorbance spectroscopy (OD_{260}) and visualized on an ethidium stained 1.2% formaldehyde gel.

Example 6: Functional expression in oocytes

Oocytes suitable for injection were obtained from adult female *Xenopus laevis* toads using procedures described in C. J. Marcus-Sekura and M. J. M. Hitchcock, *Methods in Enzymology*, Vol. 152 (1987). Pieces of ovarian lobe were incubated for 30 minutes in Ca^{2+}-free Modified Barths Saline (MBS) containing 1.5 mg/ml collagenase type IA (Worthington). Subsequently, 5 ng of RNA transcript prepared as described in Example 5, were injected into each oocyte. Following injection, oocytes were incubated at 16°C in MBS containing 0.5 mM $CaCl_2$ for 2-7 days prior to electrophysiological examination.

The ability of each chimeric receptor to function was determined by voltage-recording of current-passing electrodes across the oocyte membrane in response to glutamate and calcium receptor agonists. Oocytes were voltage clamped at a holding potential of -60 mV with an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA) using standard two electrode voltage-clamp techniques . Currents were recorded on a chart recorder. The standard control saline was MBS containing 0.3 mM $CaCl_2$ and 0.8 $MgCl_2$. Test substances were applied by superfusion at a

flow rate of about 5 ml/min. All experiments were done at room temperature. The holding current was stable in a given oocyte and varied between +10 to -200 nA for different oocytes. Activation of I_{Cl} in response to activation of receptors and subsequent increases in intracellular Ca2+ ($[Ca]_{in}$) was quantified by measuring the peak inward current stimulated by agonist or drug, relative to the holding current at -60 mV.

Figure 6 pR1/CaR vs. rat mGluR1 (glutamate and quisqualate).

Figure 7 CaR/R1 vs. hPCar (calcium)

Figure 8 pratCH3 vs. rat mGluR1 and CaR (desensitization traces)

Example 7: Construction of pCEPCaR/R1 from pCaR/R1

The DNA from plasmid pCaR/R1 was digested and cloned into the commercially available episomal mammalian expression vector, pCEP4 (Invitrogen), using the restriction enzymes Kpn I and Not I. The ligation products were transfected into DH5a cells which had been made competent for DNA transformation. These cells were plated on Luria-Bertani Media (LB) plates (described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 1989)) containing 100 ug/ml ampicillin. A clone was selected from the colonies which grew. This clone, pCEPCaR/R1 was characterized by restriction enzyme digestion.

Example 8: Transfection and growth of HEK293/ pCEPCaR/R1

Human embryonic kidney cells (293, ATCC, CRL 1573) were grown in a routine manner. Cells were plated in 10 cm cell-culture plates in Dulbecco's modified Eagle's medium (D-MEM) containing 10 % fetal calf serum (FCS) and 1 X Penicillin-Streptomycin (PS, Life Technologies) so that they would be ~70% confluent after an overnight incubation. To prepare DNA for transfection, the plasmid pCEPCaR/R1 was precipitated with ethanol, rinsed and resuspended in sterile water at a concentration of 1 ug/ul. Fourteen micrograms of DNA was incubated with the liposome formulation LipofectAMINE™ (Life Technologies) for 20 minutes in serum-free Opti-MEM® (Life Technologies). After the room temperature incubation, 6.8 mls of Opti-MEM® was added to the transfection mix. This solution was added to the cells which had been rinsed with 2X 5 ml washes of serum-free Opti-MEM®. The cells and transfection mix were incubated at 37°C for 5 hours at which time more media and fetal bovine serum were added to bring the serum concentration to 10 %. After an overnight incubation the media was changed back to D-MEM with 10% FCS and 1 x PS. After an additional 24 h incubation, cells were detached with trypsin and replated in media containing 200 ug/ml hygromycin (Boerhringer Mannheim). Those cells which grew contained pCEPCaR/R1 which encodes the hygromycin resistance gene. Individual clones were recovered and propagated using standard tissue-culture techniques. Subcultures of both individual clones and pooled stables were prepared by dissociation into fresh

tissue culture media, and plated into fresh culture dishes at 1/10th the original volume.

Example 9: HEK293/pCEPCaR/R1 Fura assay

Measurements of intracellular calcium release in response to increases in extracellular calcium is quantitated using the Fura assay (Parks et al. 1989). Stably transfected cells containing pCEPCaR/R1 are loaded with 2 μM fura-2 acetoxymethylester by incubation for 20-30 minutes at 37°C in SPF-PCB (126 mM NaCl, 5mM KCl, 1mM $MgCl_2$, 20 mM HEPES, pH 7.4), containing 1.25 mM $CaCl_2$, 1 mg/ml glucose, 0.5% BSA[1]. The cells are then washed 1 to 2 times in SPF-PCB containing 0.5 mM $CaCl_2$, 0.5% BSA and resuspended to a density of 4 to 5 million cells/ml and kept at 22°C in a plastic beaker. For recording fluorescent signals, the cells are diluted fivefold into a quartz cuvette with BSA-free 37°C SPF-PCB to achieve a final BSA concentration of 0.1% (1.2 ml of 37°C BSA-free SPF-PCB + 0.3 ml cell suspension). Measurements of fluorescence are performed at 37°C with constant stirring using a custom-built spectrofluorimeter (Biomedical Instrumentation Group, University of Pennsylvania). Excitation and emission wavelengths are 340 and 510 nm, respectively. To calibrate fluorescence signals, digitonin (Sigma, St. Louis, MO; catalog # D 5628; 50 μg/ml, final) is added to obtain F_{max} , and the apparent F_{min} is determined by adding EGTA (10 mM, final) and Tris base (pH ~ 10, final). Concentrations of released intracellular Ca^{2+}

is calculated using a dissociation constant (Kd) of 224 nM and the equation:

$$[Ca^{2+}]_i = (F - F_{min}/F_{max} - F) \times Kd$$

The results are graphically represented in Figure 9.

Example 10: Recombinant Receptor Binding Assay

The following is one example of a rapid screening assay to obtain compounds modulating metabotropic glutamate receptor activity. The screening assay first measures the ability of compounds to bind to recombinant chimeric receptors, or receptor fragments or mGluR, CaR or chimeric receptors. Compounds binding to such receptors or fragments are then tested for their ability to modulate one or more activities at a metabotropic glutamate receptor.

In one procedure, a cDNA or gene clone encoding a metabotropic glutamate receptor is obtained. Distinct fragments of the clone are expressed in an appropriate expression vector to produce the smallest receptor polypeptide(s) obtainable able to bind glutamate. Such experiments can be facilitated by utilizing a stably transfected mammalian cell line (*e.g.*, HEK 293 cells) expressing the metabotropic glutamate receptor.

The recombinant polypeptide(s) having the desired binding properties can be bound to a solid-phase support using standard chemical procedures. This solid-phase, or affinity matrix, may then be contacted with glutamate to

demonstrate that glutamate can bind to the column, and to identify conditions by which glutamate may be removed from the solid-phase. This procedure may then be repeated using a large library of compounds to determine those compounds which are able to bind to the affinity matrix. Bound compounds can then can be released in a manner similar to glutamate. Alternative binding and release conditions may be utilized to obtain compounds capable of binding under conditions distinct from those used for glutamate binding (*e.g.*, conditions which better mimic physiological conditions encountered especially in pathological states). Compounds binding to the mGluR can thus be selected from a very large collection of compounds present in a liquid medium or extract.

In an alternate method, chimeric metabotropic glutamate/calcium receptors are bound to a column or other solid phase support. Those compounds which are not competed off by reagents binding to the glutamate binding site on the receptor can then be identified. Such compounds define alternative binding sites on the receptor. Such compounds may be structurally distinct from known compounds and may define chemical classes of agonists or antagonists which may be useful as therapeutics agents.

Other embodiments are within the following claims.

CLAIMS

What we claim is:

1. A composition comprising a chimeric receptor,

wherein said chimeric receptor comprises an extracellular domain, a seven transmembrane domain, and an intracellular cytoplasmic tail domain,

wherein a sequence of at least 6 contiguous amino acids is homologous to a sequence of a metabotropic glutamate receptor, and a sequence of at least 6 contiguous amino acids is homologous to a sequence of a calcium receptor.

2. The composition of claim 1,

wherein at least one domain of said extracellular domain, said seven transmembrane domain, and said intracellular cytoplasmic tail domain is homologous to a domain of a metabotropic glutamate receptor and/or at least one domain is homologous to a domain of a calcium receptor.

3. The composition of claim 2,

wherein at least one domain of said extracellular domain, said seven transmembrane domain, and said intracellular cytoplasmic tail domain is homologous to a domain of a metabotropic glutamate receptor, and

at least one domain is homologous to a domain of a calcium receptor.

4. The composition of claim 3 wherein said chimeric receptor comprises

a domain homologous to the extracellular domain of a calcium receptor,

a domain homologous to the seven transmembrane domain of a metabotropic glutamate receptor, and

a domain homologous to the intracellular cytoplasmic tail domain of a metabotropic glutamate receptor.

5. The composition of claim 3, wherein said chimeric receptor comprises

a domain homologous to the extracellular domain of a calcium receptor,

a domain homologous to the seven transmembrane domain of a calcium receptor, and

a domain homologous to the intracellular cytoplasmic tail domain of a metabotropic glutamate receptor.

6. The composition of claim 3 wherein said chimeric receptor comprises

a domain homologous to the extracellular domain of a metabotropic glutamate receptor,

a domain homologous to the seven transmembrane domain of a calcium receptor, and

a domain homologous to the intracellular cytoplasmic tail domain of a metabotropic glutamate receptor.

7. The composition of claim 3 wherein said chimeric receptor comprises

a domain homologous to an extracellular domain of a metabotropic glutamate receptor,

a domain homologous to the seven transmembrane domain of a metabotropic glutamate receptor, and

a domain homologous to the intracellular cytoplasmic tail domain of a calcium receptor.

8. The composition of claim 3 wherein said chimeric receptor comprises

a domain homologous to the extracellular domain of a metabotropic glutamate receptor,

a domain homologous to the seven transmembrane domain of a calcium receptor, and

a domain homologous to the intracellular cytoplasmic tail domain of a calcium receptor.

9. The composition of claim 3 wherein said chimeric receptor comprises

a domain homologous to the extracellular domain of a calcium receptor,

a domain homologous to the seven transmembrane domain of a metabotropic glutamate receptor, and

a domain homologous to the intracellular cytoplasmic tail domain of a calcium receptor.

10. The composition of claim 3 wherein said chimeric receptor comprises

a domain homologous to an extracellular domain of a metabotropic glutamate receptor,

a domain homologous to the seven transmembrane domain of a metabotropic glutamate receptor except that said seven transmembrane domain comprises at least one cytoplasmic loop of a calcium receptor, and

a domain homologous to the intracellular cytoplasmic tail domain of a calcium receptor.

11. The composition of claim 1,

wherein at least one cytoplasmic loop of said seven transmembrane domain is homologous to a cytoplasmic loop of a metabotropic glutamate receptor.

12. The composition of claim 1,

wherein at least one cytoplasmic loop of said seven transmembrane domain is homologous to a cytoplasmic loop of a calcium receptor.

13. The composition of claim 1,

wherein at least a 6 contiguous amino acid sequence of said chimeric receptor is homologous to a sequence of amino acids of a calcium receptor and the remainder of the amino acid sequence of said chimeric receptor is homologous to a sequence of amino acids of a metabotropic glutamate receptor.

14. The composition of claim 1,

wherein at least a 6 contiguous amino acid sequence of said chimeric receptor is homologous to a sequence of amino acids of a metabotropic glutamate receptor and the

remainder of the amino acid sequence of said chimeric receptor is homologous to a sequence of amino acids of a calcium receptor.

15. A composition comprising an enriched, purified, or isolated nucleic acid molecule which codes for a chimeric receptor,

wherein said chimeric receptor comprises an extracellular domain, a seven transmembrane domain, and an intracellular cytoplasmic tail domain,

wherein a sequence of at least 6 contiguous amino acids is homologous to a sequence of a metabotropic glutamate receptor, and a sequence of at least 6 contiguous amino acids is homologous to a sequence of a calcium receptor.

16. The composition of claim 15,

wherein said chimeric receptor comprises

at least one domain homologous to a domain of a calcium receptor, and

at least one domain homologous to a domain of a metabotropic glutamate receptor.

17. The composition of claim 16 wherein said chimeric receptor comprises

a domain homologous to the extracellular domain of a metabotropic glutamate receptor,

a domain homologous to the seven transmembrane domain of a calcium receptor, and

a domain homologous to the intracellular cytoplasmic tail domain of a metabotropic glutamate receptor.

18. The composition of claim 16 wherein said chimeric receptor comprises

a domain homologous to an extracellular domain of a metabotropic glutamate receptor,

a domain homologous to the seven transmembrane domain of a metabotropic glutamate receptor, and

a domain homologous to the intracellular cytoplasmic tail domain of a calcium receptor.

19. The composition of claim 16 wherein said chimeric receptor comprises

a domain homologous to the extracellular domain of a metabotropic glutamate receptor,

a domain homologous to the seven transmembrane domain of a calcium receptor, and

a domain homologous to the intracellular cytoplasmic tail domain of a calcium receptor.

20. The composition of claim 16 wherein said chimeric receptor comprises

a domain homologous to an extracellular domain of a metabotropic glutamate receptor,

a domain homologous to the seven transmembrane domain of a metabotropic glutamate receptor except that said seven transmembrane domain comprises at least one cytoplasmic loop of a calcium receptor, and

a domain homologous to the intracellular cytoplasmic tail domain of a calcium receptor.

21. A composition comprising a nucleic acid coding for the chimeric receptor of claim 13.

22. A composition comprising a nucleic acid coding for the chimeric receptor of claim 14.

23. A replicable expression vector comprising a nucleic acid molecule which codes for the chimeric receptor of claim 2.

24. A recombinant host cell transformed with the vector of claim 23.

25. A process for the production of a chimeric receptor, said process comprising:

growing, under suitable nutrient conditions, procaryotic or eucaryotic host cells transformed or transfected with the expression vector of claim 13, in a manner allowing expression of said chimeric receptor.

26. A method of screening for a compound that binds to or modulates the activity of a metabotropic glutamate receptor, comprising:

a. preparing a chimeric receptor comprising an extracellular domain, a seven transmembrane domain, and an intracellular cytoplasmic tail domain wherein at least one

domain is homologous to a domain of a metabotropic glutamate receptor and at least one domain is homologous to a domain of a calcium receptor,

b. introducing said chimeric receptor and said compound into an acceptable medium, and

c. monitoring the binding or modulation by physically detectable means thereby identifying those compounds which bind to or modulate the activity of said metabotropic glutamate receptor.

27. The method of claim 26, wherein said extracellular domain of said chimeric receptor is homologous to the extracellular domain of a metabotropic glutamate receptor.

28. The method of claim 27 wherein said chimeric receptor comprises

a domain homologous to the extracellular domain of a metabotropic glutamate receptor,

a domain homologous to the seven transmembrane domain of a calcium receptor, and

a domain homologous to the intracellular cytoplasmic tail domain of a metabotropic glutamate receptor.

29. The method of claim 27 wherein said chimeric receptor comprises

a domain homologous to an extracellular domain of a metabotropic glutamate receptor,

a domain homologous to the seven transmembrane domain of a metabotropic glutamate receptor, and

a domain homologous to the intracellular cytoplasmic tail domain of a calcium receptor.

30. The method of claim 27 wherein said chimeric receptor comprises

a domain homologous to the extracellular domain of a metabotropic glutamate receptor,

a domain homologous to the seven transmembrane domain of a calcium receptor, and

a domain homologous to the intracellular cytoplasmic tail domain of a calcium receptor.

31. The method of claim 27 wherein said chimeric receptor comprises

a domain homologous to an extracellular domain of a metabotropic glutamate receptor,

a domain homologous to the seven transmembrane domain of a metabotropic glutamate receptor except that said seven transmembrane domain comprises at least one cytoplasmic loop of a calcium receptor, and

a domain homologous to the intracellular cytoplasmic tail domain of a calcium receptor.

32. A method of screening for a compound which binds to or modulates the activity of a metabotropic glutamate receptor, comprising the steps of:

a. preparing a nucleic acid sequence encoding a chimeric receptor comprising an extracellular domain, a seven transmembrane domain, and an intracellular cytoplasmic tail domain, wherein the chimeric receptor comprises a sequence of at least 6 contiguous amino acids which is homologous to a sequence of amino acids of a calcium receptor and a sequence of at least 6 contiguous amino acids which is homologous to a sequence of amino acids of a metabotropic glutamate receptor.

b. inserting the sequence into a replicable expression vector capable of expressing said chimeric receptor in a host cell,

c. transforming a host cell with the vector of (b),

d. introducing said transformed host cell and said compound into an acceptable medium, and

e. monitoring the effect of said compound on said host cell.

33. The method of claim 32, wherein said chimeric receptor comprises at least one domain homologous to a domain of a metabotropic glutamate receptor and/or at least one domain homologous to a domain of a calcium receptor.

34. The method of claim 33, wherein said chimeric receptor comprises an extracellular domain homologous to an extracellular domain of a metabotropic glutamate receptor.

35. The method of claim 34 wherein said chimeric receptor comprises

a domain homologous to the extracellular domain of a metabotropic glutamate receptor,

a domain homologous to the seven transmembrane domain of a calcium receptor, and

a domain homologous to the intracellular cytoplasmic tail domain of a metabotropic glutamate receptor.

36. The method of claim 34 wherein said chimeric receptor comprises

a domain homologous to an extracellular domain of a metabotropic glutamate receptor,

a domain homologous to the seven transmembrane domain of a metabotropic glutamate receptor, and

a domain homologous to the intracellular cytoplasmic tail domain of a calcium receptor.

37. The method of claim 34 wherein said chimeric receptor comprises

a domain homologous to the extracellular domain of a metabotropic glutamate receptor,

a domain homologous to the seven transmembrane domain of a calcium receptor, and

a domain homologous to the intracellular cytoplasmic tail domain of a calcium receptor.

38. The method of claim 34 wherein said chimeric receptor comprises

a domain homologous to an extracellular domain of a metabotropic glutamate receptor,

a domain homologous to the seven transmembrane domain of a metabotropic glutamate receptor except that said seven transmembrane domain comprises at least one cytoplasmic loop of a calcium receptor, and

a domain homologous to the intracellular cytoplasmic tail domain of a calcium receptor.

39. The method of claim 33, wherein said chimeric receptor comprises a seven transmembrane domain homologous to the seven transmembrane domain of a metabotropic glutamate receptor.

40. The method of claim 32, wherein at least one cytoplasmic loop of said seven transmembrane domain is homologous to a cytoplasmic loop of a calcium receptor.

41. The method of claim 40, wherein the sequence of the remainder of said chimeric receptor is homologous to the sequence of a metabotropic glutamate receptor.

42. The method of claim 32, wherein said chimeric receptor comprises a sequence of at least 6 contiguous amino acids which is homologous to a sequence of amino acids of a calcium receptor, and the remainder of the amino acid sequence of said chimeric receptor is homologous to a sequence of amino acids of a metabotropic glutamate receptor.

43. The method of claim 32, wherein at least one cytoplasmic loop of said seven transmembrane domain is homologous to a cytoplasmic loop of a metabotropic glutamate receptor.

44. The method of claim 32, wherein said host cell is a eukaryotic cell.

45. A method of screening for a compound that binds to a metabotropic glutamate receptor or a calcium receptor, comprising the steps of:

a. preparing a nucleic acid sequence encoding a fragment of a receptor,

b. inserting said sequence into a replicable expression vector capable of expressing said fragment in a host cell,

c. transforming a host cell with the vector of (b),

d. recovering the fragment from said host cell,

e. introducing said fragment and said compound into an acceptable medium, and

f. monitoring the binding of the compound to the fragment by physically detectable means.

46. The method of claim 45, wherein said receptor is a metabotropic glutamate receptor.

47. The method of claim 46, wherein said fragment comprises an extracellular domain of said metabotropic glutamate receptor.

48. The method of claim 46, wherein said fragment comprises a seven transmembrane domain of said metabotropic glutamate receptor.

49. The method of claim 46 wherein said fragment comprises a seven transmembrane domain and a cytoplasmic tail domain of a metabotropic glutamate receptor.

50. The method of claim 45 wherein said receptor is a calcium receptor.

51. The method of claim 50 wherein said fragment comprises an extracellular domain of said calcium receptor.

52. The method of claim 50 wherein said fragment comprises a seven transmembrane domain of said calcium receptor.

53. The method of claim 50 wherein said fragment comprises a seven transmembrane domain and a cytoplasmic tail domain of said calcium receptor.

54. A method of screening for a compound that binds to or modulates a metabotropic glutamate receptor or a calcium receptor, comprising the steps of:

a. preparing a nucleic acid sequence encoding a fragment of a receptor,

b. inserting said sequence into a replicable expression vector capable of expressing said fragment in a host cell,

c. transforming a host cell with the vector of (b),

d. introducing said transformed host cell and said compound into an acceptable medium, and

e. monitoring the effect of said compound on said host cell.

55. The method of claim 54, wherein said fragment comprises the seven transmembrane domain and cytoplasmic tail domain of a metabotropic glutamate receptor.

56. The method of claim 54, wherein said fragment comprises the seven transmembrane domain and cytoplasmic tail domain of a calcium receptor.

57. A method of screening for a compound that binds to or modulates a receptor, comprising the steps of:

a. preparing a nucleic acid sequence encoding a first fragment comprising a fragment of a first receptor,

b. inserting the sequence into a replicable expression vector capable of expressing said first fragment in a host cell,

c. transforming a host cell with the vector of (b),

d. recovering the first fragment from the host cell,

e. preparing a nucleic acid sequence encoding a second fragment comprising a fragment of a second receptor,

f. inserting the sequence of (e) into a replicable expression vector capable of expressing said second fragment in a host cell,

g. transforming a host cell with the vector of (f),

h. recovering the second fragment from the host cell of (g), and

i. introducing said first fragment and said second fragment and said compound into an acceptable medium, and

j. monitoring the binding and/or modulation of the compound by physically detectable means.

58. The method of claim 57, wherein

said first fragment comprises the extracellular domain of a metabotropic glutamate receptor, and

said second fragment comprises the seven transmembrane domain and the cytoplasmic tail domain of a calcium receptor.

59. The method of claim 57, wherein

said first fragment comprises the extracellular domain and the seven transmembrane domain of a metabotropic glutamate receptor, and

said second fragment comprises the cytoplasmic tail domain of a calcium receptor.

60. The method of claim 57 wherein

said first fragment comprises the extracellular domain of a calcium receptor, and

said second fragment comprises the seven transmembrane domain and the cytoplasmic tail domain of a metabotropic glutamate receptor.

61. The method of claim 57 wherein

said first fragment comprises the extracellular domain of a calcium receptor, and

said second fragment comprises the seven transmembrane domain of a metabotropic glutamate receptor and the cytoplasmic tail domain of a calcium receptor.

62. A method of screening for compounds which modulate the activity of both a metabotropic glutamate receptor and a calcium receptor, comprising the steps of:

a. preparing a nucleic acid sequence encoding a chimeric receptor wherein the chimeric receptor comprises an extracellular domain, a seven transmembrane domain, and an intracellular cytoplasmic tail domain, wherein at least one domain is homologous to a domain of the metabotropic glutamate receptor and at least one domain is homologous to a domain of a calcium receptor.

b) inserting the sequence into a replicable expression vector capable of expressing said chimeric receptor in a host cell,

c) transforming a host cell with the vector of (b),

d) introducing said transformed host cell and said compound into an acceptable medium, and

e) monitoring the effect of said compound on said cell.

63. A method of determining the site-of-action of a metabotropic glutamate receptor active compound, comprising the steps of:

a. preparing a nucleic acid sequence encoding a chimeric receptor wherein the chimeric receptor comprises at least a 6 amino acid sequence which is homologous to a sequence of amino acids of a calcium receptor and the remainder of the amino acid sequence is homologous to a sequence of amino acids of a metabotropic glutamate receptor,

b. inserting the sequence into a replicable expression vector capable of expressing said chimeric receptor in a host cell,

c. transforming a host cell with the vector of (b),

d. introducing said transformed host cell and said compound into an acceptable medium, and

e. monitoring the effect of said compound on said cell.

64. A method of determining the site-of-action of a calcium receptor active compound, comprising the steps of:

a. preparing a nucleic acid sequence encoding a chimeric receptor wherein the chimeric receptor comprises at least a 6 amino acid sequence which is homologous to a sequence of amino acids of a metabotropic glutamate

receptor and the remainder of the amino acid sequence is homologous to a sequence of amino acids of a calcium receptor,

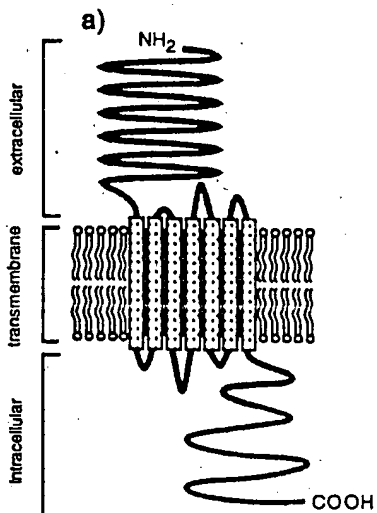
b. inserting the sequence into a replicable expression vector capable of expressing said chimeric receptor in a host cell,

c. transforming a host cell with the vector of (b),

d. introducing said transformed host cell and said compound into an acceptable medium, and

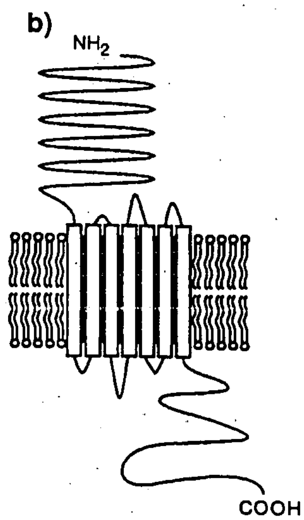
e. monitoring the effect of said compound on said cell.

FIG. 1a.



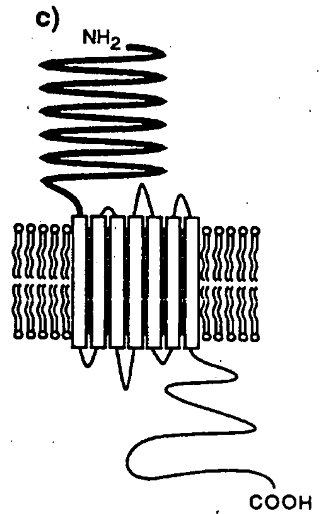
mGluR

FIG. 1b.



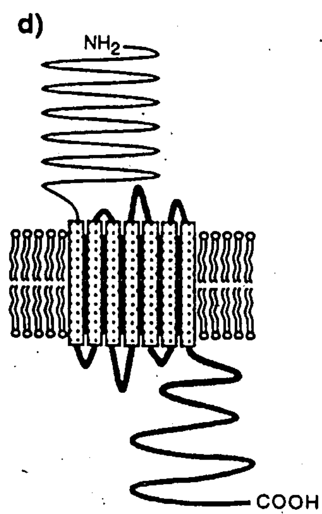
CaR

FIG. 1c.



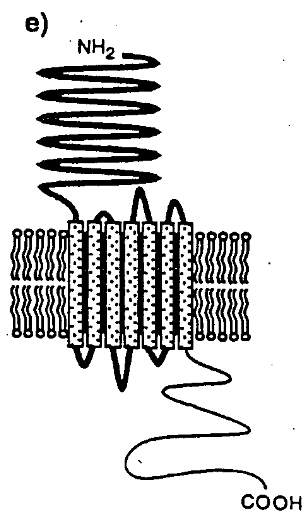
mGluR/CaR
($mGluR_{ex}$/CaR)

FIG. 1d.



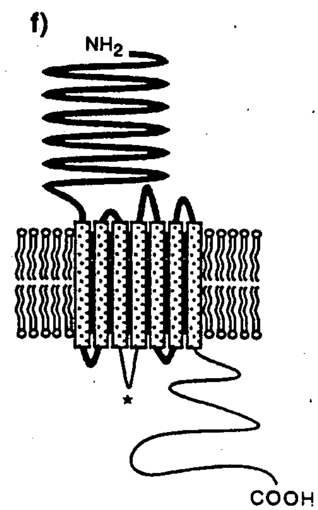
CaR/mGluR
(CaR_{ex}/mGluR)

FIG. 1e.



CH3
(mGluR/CaR_{in})

FIG. 1f.



CH3-2*
(mGluR/CaR_{in})

FIG. 2a.

Sequence Range: -7 to 3379

```
           3                13                 23                 33
     *     *         *          *         *          *          *          *
CGCCACA ATG GTC CGG CTC CTC TTG ATT TTC TTC CCA ATG ATC TTT TTG
        Met Val Arg Leu Leu Leu Ile Phe Phe Pro Met Ile Phe Leu>
            b   CODING SEQUENCE CHIMERA:JUNCTION NUC.1776     b   >
        a     a     a   -8 TO 1775 OF MCRATMGL-1      30   a     a     a40 >

43              53               63               73               83
 *      *        *        *        *        *        *        *        *        *
GAG ATG TCC ATT TTG CCC AGG ATG CCT GAC AGA AAA GTA TTG CTG GCA
Glu Met Ser Ile Leu Pro Arg Met Pro Asp Arg Lys Val Leu Leu Ala>
   b    b   CODING SEQUENCE CHIMERA:JUNCTION NUC.1776     b    b   >
   a    a 50a    a    -8 TO 1775 OF MCRATMGL-1      a 80a    a    a   >

 93              103              113              123              133
  *        *        *        *        *        *        *        *        *        *
GGT GCC TCG TCC CAG CGC TCC GTG GCG AGA ATG GAC GGA GAT GTC ATC
Gly Ala Ser Ser Gln Arg Ser Val Ala Arg Met Asp Gly Asp Val Ile>
   b    b   CODING SEQUENCE CHIMERA:JUNCTION NUC.1776     b    b   >
90  a    a    100 a  -8 TO 1775 OF MCRATMGL-1 a    a    130 a    a   >

   143             153              163              173              183
     *        *        *        *        *        *        *        *        *
ATC GGA GCC CTC TTC TCA GTC CAT CAC CAG CCT CCA GCC GAG AAG GTA
Ile Gly Ala Leu Phe Ser Val His His Gln Pro Pro Ala Glu Lys Val>
   b    b   CODING SEQUENCE CHIMERA:JUNCTION NUC.1776     b    b   >
140a    a    a   150   -8 TO 1775 OF MCRATMGL-1      a    a   180   a   >

        193              203              213              223              233
  *        *        *        *        *        *        *        *        *        *
CCC GAA AGG AAG TGT GGG GAG ATC AGG GAA CAG TAT GGT ATC CAG AGG
Pro Glu Arg Lys Cys Gly Glu Ile Arg Glu Gln Tyr Gly Ile Gln Arg>
   b    b   CODING SEQUENCE CHIMERA:JUNCTION NUC.1776     b    b   >
   190 a    a    a20 -8 TO 1775 OF MCRATMGL-1 20 a    a    a230a   >

          243              253              263              273
     *        *        *        *        *        *        *        *        *
GTG GAG GCC ATG TTC CAC ACG TTG GAT AAG ATT AAC GCG GAC CCG GTG
Val Glu Ala Met Phe His Thr Leu Asp Lys Ile Asn Ala Asp Pro Val>
   b    b   CODING SEQUENCE CHIMERA:JUNCTION NUC.1776     b    b   >
   a   240   a    a    -8 TO 1775 OF MCRATMGL-1   270   a    a    280 >

283             293              303              313              323
 *      *        *        *        *        *        *        *        *        *
CTC CTG CCC AAC ATC ACT CTG GGC AGT GAG ATC CGG GAC TCC TGC TGG
Leu Leu Pro Asn Ile Thr Leu Gly Ser Glu Ile Arg Asp Ser Cys Trp>
   b    b   CODING SEQUENCE CHIMERA:JUNCTION NUC.1776     b    b   >
   a    a290a    a    -8 TO 1775 OF MCRATMGL-1      a320a    a    a   >

333             343              353              363              373
  *        *        *        *        *        *        *        *        *        *
CAC TCT TCA GTG GCT CTC GAA CAG AGC ATC GAA TTC ATC AGA GAC TCC
His Ser Ser Val Ala Leu Glu Gln Ser Ile Glu Phe Ile Arg Asp Ser>
   b    b   CODING SEQUENCE CHIMERA:JUNCTION NUC.1776     b    b   >
330   a    a    340 a  -8 TO 1775 OF MCRATMGL-1 a    a    370 a    a   >

   383             393              403              413              423
     *        *        *        *        *        *        *        *        *
CTG ATT TCC ATC CGA GAT GAG AAG GAT GGG CTG AAC CGA TGC CTG CCT
Leu Ile Ser Ile Arg Asp Glu Lys Asp Gly Leu Asn Arg Cys Leu Pro>
```

FIG. 2b.

```
   b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
380a    a    a  390  -8 TO 1775 OF MCRATMGL-1     a    a  420  a   >

      433            443            453             463            473
 *        *       *        *        *       *        *       *       *       *
GAT GGC CAG ACC CTG CCC CCT GGC AGG ACT AAG AAG CCT ATT GCT GGA
Asp Gly Gln Thr Leu Pro Pro Gly Arg Thr Lys Lys Pro Ile Ala Gly>
   b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
   430 a    a    a44 -8 TO 1775 OF MCRATMGL-1 60 a    a    a470a   >

        483             493             503            513
    *        *        *        *        *        *        *        *        *
GTG ATC GGC CCT GGC TCC AGC TCT GTG GCC ATT CAA GTC CAG AAT CTT
Val Ile Gly Pro Gly Ser Ser Ser Val Ala Ile Gln Val Gln Asn Leu>
   b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
   a  480   a    a    -8 TO 1775 OF MCRATMGL-1    510   a    a   520 >

523          533            543             553            563
 *        *        *        *        *        *        *        *        *        *
CTC CAG CTG TTC GAC ATC CCA CAG ATC GCC TAT TCT GCC ACA AGC ATA
Leu Gln Leu Phe Asp Ile Pro Gln Ile Ala Tyr Ser Ala Thr Ser Ile>
   b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
   a    a530a    a    -8 TO 1775 OF MCRATMGL-1    a560a    a    a   >

 573             583            593             603            613
   *        *        *        *        *        *        *        *        *        *
GAC CTG AGT GAC AAA ACT TTG TAC AAA TAC TTC CTG AGG GTG GTC CCT
Asp Leu Ser Asp Lys Thr Leu Tyr Lys Tyr Phe Leu Arg Val Val Pro>
   b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
570  a    a    580 a   -8 TO 1775 OF MCRATMGL-1 a    a    610 a    a   >

    623            633             643             653            663
      *        *        *        *        *        *        *        *        *
TCT GAC ACT TTG CAG GCA AGG GCG ATG CTC GAC ATA GTC AAG CGT TAC
Ser Asp Thr Leu Gln Ala Arg Ala Met Leu Asp Ile Val Lys Arg Tyr>
   b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
620a    a    a  630  -8 TO 1775 OF MCRATMGL-1     a    a  660  a   >

      673            683             693             703            713
 *        *        *        *        *        *        *        *        *        *
AAC TGG ACC TAT GTC TCA GCA GTC CAC ACA GAA GGG AAT TAC GGC GAG
Asn Trp Thr Tyr Val Ser Ala Val His Thr Glu Gly Asn Tyr Gly Glu>
   b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
   670 a    a    a68 -8 TO 1775 OF MCRATMGL-1 00 a    a    a710a   >

        723             733            743             753
    *        *        *        *        *        *        *        *        *
AGT GGA ATG GAT GCT TTC AAA GAA CTG GCT GCC CAG GAA GGC CTC TGC
Ser Gly Met Asp Ala Phe Lys Glu Leu Ala Ala Gln Glu Gly Leu Cys>
   b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
   a  720   a    a    -8 TO 1775 OF MCRATMGL-1    750   a    a   760 >

763          773            783             793            803
 *        *        *        *        *        *        *        *        *        *
ATC GCA CAC TCG GAC AAA ATC TAC AGC AAT GCT GGC GAG AAG AGC TTT
Ile Ala His Ser Asp Lys Ile Tyr Ser Asn Ala Gly Glu Lys Ser Phe>
   b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
   a    a770a    a    -8 TO 1775 OF MCRATMGL-1    a800a    a    a   >

 813             823            833             843            853
   *        *        *        *        *        *        *        *        *        *
GAC CGG CTC CTG CGT AAA CTC CGG GAG CGG CTT CCC AAG GCC AGG GTT
```

FIG. 2c.

```
 Asp Arg Leu Leu Arg Lys Leu Arg Glu Arg Leu Pro Lys Ala Arg Val>
    b   b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b   b   >
810 a   a   820 a  -8 TO 1775 OF MCRATMGL-1 a   a   850 a   a   >

    863         873         883         893         903
     *   *   *   *   *   *   *   *   *
 GTG GTC TGC TTC TGC GAG GGC ATG ACA GTG CGG GGC TTA CTG AGT GCC
 Val Val Cys Phe Cys Glu Gly Met Thr Val Arg Gly Leu Leu Ser Ala>
    b   b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b   b   >
 860a   a   a 870  -8 TO 1775 OF MCRATMGL-1    a   a  900  a   >

      913         923         933         943         953
  *   *   *   *   *   *   *   *   *
 ATG CGC CGC CTG GGC GTC GTG GGC GAG TTC TCA CTC ATT GGA AGT GAT
 Met Arg Arg Leu Gly Val Val Gly Glu Phe Ser Leu Ile Gly Ser Asp>
    b   b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b   b   >
   910 a   a   a92 -8 TO 1775 OF MCRATMGL-1 40 a   a   a950a    >

       963         973         983         993
     *   *   *   *   *   *   *   *   *
 GGA TGG GCA GAC AGA GAT GAA GTC ATC GAA GGC TAT GAG GTG GAA GCC
 Gly Trp Ala Asp Arg Asp Glu Val Ile Glu Gly Tyr Glu Val Glu Ala>
    b   b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b   b   >
    a  960  a   a   -8 TO 1775 OF MCRATMGL-1   990  a   a  1000 >

1003        1013        1023        1033        1043
  *   *   *   *   *   *   *   *   *   *
 AAC GGA GGG ATC ACA ATA AAG CTT CAG TCT CCA GAG GTC AGG TCA TTT
 Asn Gly Gly Ile Thr Ile Lys Leu Gln Ser Pro Glu Val Arg Ser Phe>
    b   b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b   b   >
    a  1010a   a   -8 TO 1775 OF MCRATMGL-1    1040a   a   a   >

  1053        1063        1073        1083        1093
   *   *   *   *   *   *   *   *   *   *
 GAT GAC TAC TTC CTG AAG CTG AGG CTG GAC ACC AAC ACA AGG AAT CCT
 Asp Asp Tyr Phe Leu Lys Leu Arg Leu Asp Thr Asn Thr Arg Asn Pro>
    b   b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b   b   >
1050 a   a   1060 a -8 TO 1775 OF MCRATMGL-1  a   a  1090 a   a   >

   1103        1113        1123        1133        1143
     *   *   *   *   *   *   *   *   *
 TGG TTC CCT GAG TTC TGG CAA CAT CGC TTC CAG TGT CGC CTA CCT GGA
 Trp Phe Pro Glu Phe Trp Gln His Arg Phe Gln Cys Arg Leu Pro Gly>
    b   b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b   b   >
  1100a   a   a 1110 -8 TO 1775 OF MCRATMGL-1 a   a   a 1140  a   >

      1153        1163        1173        1183        1193
  *   *   *   *   *   *   *   *   *   *
 CAC CTC TTG GAA AAC CCC AAC TTT AAG AAA GTG TGC ACA GGA AAT GAA
 His Leu Leu Glu Asn Pro Asn Phe Lys Lys Val Cys Thr Gly Asn Glu>
    b   b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b   b   >
  1150 a   a   116 -8 TO 1775 OF MCRATMGL-1 80 a   a   1190a    >

       1203        1213        1223        1233
     *   *   *   *   *   *   *   *   *
 AGC TTG GAA GAA AAC TAT GTC CAG GAC AGC AAA ATG GGA TTT GTC ATC
 Ser Leu Glu Glu Asn Tyr Val Gln Asp Ser Lys Met Gly Phe Val Ile>
    b   b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b   b   >
    a 1200  a   a   -8 TO 1775 OF MCRATMGL-1  1230  a   a  1240 >

1243        1253        1263        1273        1283
  *   *   *   *   *   *   *   *   *   *
```

FIG. 2d

```
AAT GCC ATC TAT GCC ATG GCA CAT GGG CTG CAG AAC ATG CAC CAT GCT
Asn Ala Ile Tyr Ala Met Ala His Gly Leu Gln Asn Met His His Ala>
   b   b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b   b   >
   a   1250a   a   -8 TO 1775 OF MCRATMGL-1    1280a   a   a   >

1293       1303       1313       1323       1333
   *    *    *    *    *    *    *    *    *    *
CTG TGT CCC GGC CAT GTG GGC CTG TGT GAT GCT ATG AAA CCC ATT GAT
Leu Cys Pro Gly His Val Gly Leu Cys Asp Ala Met Lys Pro Ile Asp>
   b   b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b   b   >
1290  a   a  1300 a -8 TO 1775 OF MCRATMGL-1  a   a  1330 a   a   >

   1343       1353       1363       1373       1383
   *    *    *    *    *    *    *    *    *    *
GGC AGG AAG CTC CTG GAT TTC CTC ATC AAA TCC TCT TTT GTC GGA GTG
Gly Arg Lys Leu Leu Asp Phe Leu Ile Lys Ser Ser Phe Val Gly Val>
   b   b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b   b   >
1340a   a   a 1350 -8 TO 1775 OF MCRATMGL-1 a   a   a 1380  a   >

      1393       1403       1413       1423       1433
   *    *    *    *    *    *    *    *    *    *
TCT GGA GAG GAG GTG TGG TTC GAT GAG AAG GGG GAT GCT CCC GGA AGG
Ser Gly Glu Glu Val Trp Phe Asp Glu Lys Gly Asp Ala Pro Gly Arg>
   b   b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b   b   >
 1390 a   a   140 -8 TO 1775 OF MCRATMGL-1 20 a   a   1430a   >

       1443       1453       1463       1473
   *    *    *    *    *    *    *    *    *
TAT GAC ATT ATG AAT CTG CAG TAC ACA GAA GCT AAT CGC TAT GAC TAT
Tyr Asp Ile Met Asn Leu Gln Tyr Thr Glu Ala Asn Arg Tyr Asp Tyr>
   b   b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b   b   >
   a 1440  a   a   -8 TO 1775 OF MCRATMGL-1  1470  a   a  1480 >

1483       1493       1503       1513       1523
   *    *    *    *    *    *    *    *    *    *
GTC CAC GTG GGG ACC TGG CAT GAA GGA GTG CTG AAT ATT GAT GAT TAC
Val His Val Gly Thr Trp His Glu Gly Val Leu Asn Ile Asp Asp Tyr>
   b   b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b   b   >
   a   1490a   a   -8 TO 1775 OF MCRATMGL-1    1520a   a   a   >

 1533       1543       1553       1563       1573
   *    *    *    *    *    *    *    *    *    *
AAA ATC CAG ATG AAC AAA AGC GGA ATG GTA CGA TCT GTG TGC AGT GAG
Lys Ile Gln Met Asn Lys Ser Gly Met Val Arg Ser Val Cys Ser Glu>
   b   b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b   b   >
1530  a   a  1540 a -8 TO 1775 OF MCRATMGL-1  a   a  1570 a   a   >

   1583       1593       1603       1613       1623
   *    *    *    *    *    *    *    *    *
CCT TGC TTA AAG GGT CAG ATT AAG GTC ATA CGG AAA GGA GAA GTG AGC
Pro Cys Leu Lys Gly Gln Ile Lys Val Ile Arg Lys Gly Glu Val Ser>
   b   b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b   b   >
1580a   a   a 1590 -8 TO 1775 OF MCRATMGL-1 a   a   a 1620  a   >

      1633       1643       1653       1663       1673
   *    *    *    *    *    *    *    *    *    *
TGC TGC TGG ATC TGC ACG GCC TGC AAA GAG AAT GAG TTT GTG CAG GAC
Cys Cys Trp Ile Cys Thr Ala Cys Lys Glu Asn Glu Phe Val Gln Asp>
   b   b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b   b   >
  1630 a   a   164 -8 TO 1775 OF MCRATMGL-1 60 a   a   1670a   >

       1683       1693       1703       1713
```

FIG. 2e.

```
      *      *       *       *      *       *       *      *       *
  GAG TTC ACC TGC AGA GCC TGT GAC CTG GGG TGG TGG CCC AAC GCA GAG
  Glu Phe Thr Cys Arg Ala Cys Asp Leu Gly Trp Trp Pro Asn Ala Glu>
     b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
     a 1680   a    a    -8 TO 1775 OF MCRATMGL-1  1710  a    a  1720 >

1723          1733          1743          1753          1763
   *      *       *       *      *       *       *      *       *       *
  CTC ACA GGC TGT GAG CCC ATT CCT GTC CGT TAT CTT GAG TGG AGT GAC
  Leu Thr Gly Cys Glu Pro Ile Pro Val Arg Tyr Leu Glu Trp Ser Asp>
     b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
     a    1730a    a    -8 TO 1775 OF MCRATMGL-1    1760a    a    a   >

  1773          1783          1793          1803          1813
     *      *       *       *      *       *       *      *       *       *
  ATA GAA GGG ATC GCA CTC ACC CTC TTT GCC GTG CTG GGC ATT TTC CTG
  Ile Glu Gly Ile Ala Leu Thr Leu Phe Ala Val Leu Gly Ile Phe Leu>
     b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
1770  a    >
            1840  c  1837 TO 3437 OF MCPHUPCAR4.0 FINAL    c    c   >

    1823          1833          1843          1853          1863
      *      *       *       *      *       *       *      *       *
  ACA GCC TTT GTG CTG GGT GTG TTT ATC AAG TTC CGC AAC ACA CCC ATT
  Thr Ala Phe Val Leu Gly Val Phe Ile Lys Phe Arg Asn Thr Pro Ile>
     b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
 1880 c    c    1 1837 TO 3437 OF MCPHUPCAR4.0 FINAL    1920c    c   >

       1873          1883          1893          1903          1913
  *      *      *       *      *       *       *      *       *       *
  GTC AAG GCC ACC AAC CGA GAG CTC TCC TAC CTC CTC CTC TTC TCC CTG
  Val Lys Ala Thr Asn Arg Glu Leu Ser Tyr Leu Leu Leu Phe Ser Leu>
     b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
   1930   c    c   1837 TO 3437 OF MCPHUPCAR4.0 FINAL   c  1970 c   >

         1923          1933          1943          1953
      *      *       *       *      *       *       *      *       *
  CTC TGC TGC TTC TCC AGC TCC CTG TTC TTC ATC GGG GAG CCC CAG GAC
  Leu Cys Cys Phe Ser Ser Ser Leu Phe Phe Ile Gly Glu Pro Gln Asp>
     b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
     1980c    c  1837 TO 3437 OF MCPHUPCAR4.0 FINAL    c    c 2020  >

1963          1973          1983          1993          2003
   *      *       *       *      *       *       *      *       *       *
  TGG ACG TGC CGC CTG CGC CAG CCG GCC TTT GGC ATC AGC TTC GTG CTC
  Trp Thr Cys Arg Leu Arg Gln Pro Ala Phe Gly Ile Ser Phe Val Leu>
     b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
     c  2030 c   1837 TO 3437 OF MCPHUPCAR4.0 FINAL 0 c    c    2070>

  2013          2023          2033          2043          2053
     *      *       *       *      *       *       *      *       *       *
  TGC ATC TCA TGC ATC CTG GTG AAA ACC AAC CGT GTC CTC CTG GTG TTT
  Cys Ile Ser Cys Ile Leu Val Lys Thr Asn Arg Val Leu Leu Val Phe>
     b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
     c    c 2080 1837 TO 3437 OF MCPHUPCAR4.0 FINAL 2110  c    c   >

    2063          2073          2083          2093          2103
      *      *       *       *      *       *       *      *       *
  GAG GCC AAG ATC CCC ACC AGC TTC CAC CGC AAG TGG TGG GGG CTC AAC
  Glu Ala Lys Ile Pro Thr Ser Phe His Arg Lys Trp Trp Gly Leu Asn>
     b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
 2120 c    c    2 1837 TO 3437 OF MCPHUPCAR4.0 FINAL    2160c    c   >
```

FIG. 2f

```
     2113        2123        2133        2143        2153
 *     *     *     *     *     *     *     *     *     *
CTG CAG TTC CTG CTG GTT TTC CTC TGC ACC TTC ATG CAG ATT GTC ATC
Leu Gln Phe Leu Leu Val Phe Leu Cys Thr Phe Met Gln Ile Val Ile>
  b   b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b   b   >
 2170 c   c  1837 TO 3437 OF MCPHUPCAR4.0 FINAL   c  2210 c   >

       2163        2173        2183        2193
   *     *     *     *     *     *     *     *     *
TGT GTG ATC TGG CTC TAC ACC GCG CCC CCC TCA AGC TAC CGC AAC CAG
Cys Val Ile Trp Leu Tyr Thr Ala Pro Pro Ser Ser Tyr Arg Asn Gln>
  b   b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b   b   >
  2220c   c  1837 TO 3437 OF MCPHUPCAR4.0 FINAL   c   c 2260  >

2203        2213        2223        2233        2243
 *     *     *     *     *     *     *     *     *     *
GAG CTG GAG GAT GAG ATC ATC TTC ATC ACG TGC CAC GAG GGC TCC CTC
Glu Leu Glu Asp Glu Ile Ile Phe Ile Thr Cys His Glu Gly Ser Leu>
  b   b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b   b   >
  c  2270 c  1837 TO 3437 OF MCPHUPCAR4.0 FINAL 0 c   c   2310>

 2253        2263        2273        2283        2293
   *     *     *     *     *     *     *     *     *     *
ATG GCC CTG GGC TTC CTG ATC GGC TAC ACC TGC CTG CTG GCT GCC ATC
Met Ala Leu Gly Phe Leu Ile Gly Tyr Thr Cys Leu Leu Ala Ala Ile>
  b   b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b   b   >
  c   c 2320 1837 TO 3437 OF MCPHUPCAR4.0 FINAL 2350  c   c   >

   2303        2313        2323        2333        2343
     *     *     *     *     *     *     *     *     *     *
TGC TTC TTC TTT GCC TTC AAG TCC CGG AAG CTG CCG GAG AAC TTC AAT
Cys Phe Phe Phe Ala Phe Lys Ser Arg Lys Leu Pro Glu Asn Phe Asn>
  b   b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b   b   >
2360 c   c   2 1837 TO 3437 OF MCPHUPCAR4.0 FINAL    2400c   c   >

      2353        2363        2373        2383        2393
 *     *     *     *     *     *     *     *     *     *
GAA GCC AAG TTC ATC ACC TTC AGC ATG CTC ATC TTC TTC ATC GTC TGG
Glu Ala Lys Phe Ile Thr Phe Ser Met Leu Ile Phe Phe Ile Val Trp>
  b   b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b   b   >
 2410  c   c  1837 TO 3437 OF MCPHUPCAR4.0 FINAL   c  2450 c   >

       2403        2413        2423        2433
   *     *     *     *     *     *     *     *     *
ATC TCC TTC ATT CCA GCC TAT GCC AGC ACC TAT GGC AAG TTT GTC TCT
Ile Ser Phe Ile Pro Ala Tyr Ala Ser Thr Tyr Gly Lys Phe Val Ser>
  b   b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b   b   >
  2460c   c  1837 TO 3437 OF MCPHUPCAR4.0 FINAL   c   c 2500  >

2443        2453        2463        2473        2483
 *     *     *     *     *     *     *     *     *     *
GCC GTA GAG GTG ATT GCC ATC CTG GCA GCC AGC TTT GGC TTG CTG GCG
Ala Val Glu Val Ile Ala Ile Leu Ala Ala Ser Phe Gly Leu Leu Ala>
  b   b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b   b   >
  c  2510 c  1837 TO 3437 OF MCPHUPCAR4.0 FINAL 0 c   c   2550>

 2493        2503        2513        2523        2533
 *     *     *     *     *     *     *     *     *     *
TGC ATC TTC TTC AAC AAG ATC TAC ATC ATT CTC TTC AAG CCA TCC CGC
Cys Ile Phe Phe Asn Lys Ile Tyr Ile Ile Leu Phe Lys Pro Ser Arg>
  b   b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b   b   >
```

FIG. 2g.

```
    c    c 2560 1837 TO 3437 OF MCPHUPCAR4.0 FINAL 2590  c    c    >

   2543          2553          2563          2573          2583
      *        *      *      *      *      *      *      *      *
  AAC ACC ATC GAG GAG GTG CGT TGC AGC ACC GCA GCT CAC GCT TTC AAG
  Asn Thr Ile Glu Glu Val Arg Cys Ser Thr Ala Ala His Ala Phe Lys>
    b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
2600 c    c    2 1837 TO 3437 OF MCPHUPCAR4.0 FINAL   2640c   c   >

      2593         2603          2613          2623          2633
  *       *      *      *      *      *      *      *      *      *
  GTG GCT GCC CGG GCC ACG CTG CGC CGC AGC AAC GTC TCC CGC AAG CGG
  Val Ala Ala Arg Ala Thr Leu Arg Arg Ser Asn Val Ser Arg Lys Arg>
    b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
  2650   c    c   1837 TO 3437 OF MCPHUPCAR4.0 FINAL   c  2690 c   >

        2643          2653          2663          2673
     *      *      *      *      *      *      *      *      *
  TCC AGC AGC CTT GGA GGC TCC ACG GGA TCC ACC CCC TCC TCC TCC ATC
  Ser Ser Ser Leu Gly Gly Ser Thr Gly Ser Thr Pro Ser Ser Ser Ile>
    b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
    2700c    c  1837 TO 3437 OF MCPHUPCAR4.0 FINAL   c    c 2740  >

2683          2693          2703          2713          2723
  *      *      *      *      *      *      *      *      *      *
  AGC AGC AAG AGC AAC AGC GAA GAC CCA TTC CCA CAG CCC GAG AGG CAG
  Ser Ser Lys Ser Asn Ser Glu Asp Pro Phe Pro Gln Pro Glu Arg Gln>
    b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
    c  2750 c  1837 TO 3437 OF MCPHUPCAR4.0 FINAL 0 c    c    2790>

 2733          2743          2753          2763          2773
    *      *      *      *      *      *      *      *      *      *
  AAG CAG CAG CAG CCG CTG GCC CTA ACC CAG CAA GAG CAG CAG CAG CAG
  Lys Gln Gln Gln Pro Leu Ala Leu Thr Gln Gln Glu Gln Gln Gln Gln>
    b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
    c    c 2800 1837 TO 3437 OF MCPHUPCAR4.0 FINAL 2830  c    c   >

   2783          2793          2803          2813          2823
      *      *      *      *      *      *      *      *      *
  CCC CTG ACC CTC CCA CAG CAG CAA CGA TCT CAG CAG CAG CCC AGA TGC
  Pro Leu Thr Leu Pro Gln Gln Gln Arg Ser Gln Gln Gln Pro Arg Cys>
    b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
2840 c    c    2 1837 TO 3437 OF MCPHUPCAR4.0 FINAL   2880c   c   >

      2833         2843          2853          2863          2873
  *       *      *      *      *      *      *      *      *      *
  AAG CAG AAG GTC ATC TTT GGC AGC GGC ACG GTC ACC TTC TCA CTG AGC
  Lys Gln Lys Val Ile Phe Gly Ser Gly Thr Val Thr Phe Ser Leu Ser>
    b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
  2890   c    c   1837 TO 3437 OF MCPHUPCAR4.0 FINAL   c  2930 c   >

        2883          2893          2903          2913
     *      *      *      *      *      *      *      *      *
  TTT GAT GAG CCT CAG AAG AAC GCC ATG GCC CAC GGG AAT TCT ACG CAC
  Phe Asp Glu Pro Gln Lys Asn Ala Met Ala His Gly Asn Ser Thr His>
    b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
    2940c    c  1837 TO 3437 OF MCPHUPCAR4.0 FINAL   c    c 2980  >

2923          2933          2943          2953          2963
  *      *      *      *      *      *      *      *      *      *
  CAG AAC TCC CTG GAG GCC CAG AAA AGC AGC GAT ACG CTG ACC CGA CAC
  Gln Asn Ser Leu Glu Ala Gln Lys Ser Ser Asp Thr Leu Thr Arg His>
```

FIG. 2h.

```
      b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
      c  2990 c   1837 TO 3437 OF MCPHUPCAR4.0 FINAL 0 c    c    3030>

  2973           2983           2993           3003           3013
    *       *       *       *       *       *       *       *       *       *
 CAG CCA TTA CTC CCG CTG CAG TGC GGG GAA ACG GAC TTA GAT CTG ACC
 Gln Pro Leu Leu Pro Leu Gln Cys Gly Glu Thr Asp Leu Asp Leu Thr>
      b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
      c    c 3040 1837 TO 3437 OF MCPHUPCAR4.0 FINAL 3070  c    c   >

     3023           3033           3043           3053           3063
      *       *       *       *       *       *       *       *       *
 GTC CAG GAA ACA GGT CTG CAA GGA CCT GTG GGT GGA GAC CAG CGG CCA
 Val Gln Glu Thr Gly Leu Gln Gly Pro Val Gly Gly Asp Gln Arg Pro>
      b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
 3080 c    c   3 1837 TO 3437 OF MCPHUPCAR4.0 FINAL    3120c    c   >

        3073           3083           3093           3103           3113
   *       *       *       *       *       *       *       *       *       *
 GAG GTG GAG GAC CCT GAA GAG TTG TCC CCA GCA CTT GTA GTG TCC AGT
 Glu Val Glu Asp Pro Glu Glu Leu Ser Pro Ala Leu Val Val Ser Ser>
      b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
   3130   c    c   1837 TO 3437 OF MCPHUPCAR4.0 FINAL    c  3170 c   >

           3123           3133           3143           3153
    *       *       *       *       *       *       *       *       *
 TCA CAG AGC TTT GTC ATC AGT GGT GGA GGC AGC ACT GTT ACA GAA AAC
 Ser Gln Ser Phe Val Ile Ser Gly Gly Gly Ser Thr Val Thr Glu Asn>
      b    b  CODING SEQUENCE CHIMERA:JUNCTION NUC.1776    b    b   >
      3180c    c   1837 TO 3437 OF MCPHUPCAR4.0 FINAL    c    c 3220  >

3163           3173           3183           3193           3203           3213
  *       *       *       *       *       *       *       *       *       *
 GTA GTG AAT TCA T AAAATGGA AGGAGAAGAC TGGGCTAGGG AGAATGCAGA
 Val Val Asn Ser Xxx>
      CODING SEQ   b >
      c  3230  1837 TO 3437 OF MCPHUPCAR4.0 FINAL  c    3270    >

        3223           3233           3243           3253           3263           3273
      *       *       *       *       *       *       *       *       *       *       *       *
 GAGGTTTCTT GGGGTCCCAG GGATGAGGAA TCGCCCCAGA CTCCTTTCCT CTGAGGAAGA
      3280    c      1837 TO 3437 OF MCPHUPCAR4.0 FINAL 0    c    3330   >

        3283           3293           3303           3313           3323           3333
      *       *       *       *       *       *       *       *       *       *       *       *
 AGGGATAATA GACACATCAA ATGCCCCGAA TTTAGTCACA CCATCTTAAA TGACAGTGAA
      3340   c      1837 TO 3437 OF MCPHUPCAR4.0 FINAL 0    c    3390   >

        3343           3353           3363           3373
      *       *       *       *       *       *       *       *       *
 TTGACCCATG TTCCCTTTAA AAAAAAAAAA AAAAAAGCGG CCGC--
      34 1837 TO 3437 OF MCPHUPCAR4.0 FINAL    c    >
```

FIG. 3a.

```
Sequence Range: -40 to 3960

          -31       -21       -11        -1              10
        *     *     *    *     *    *    *    *      *      *      *
CTAGCTGTCT CATCCCTTGC CCTGGAGAGA CGGCAGAACC ATG GCA TTT TAT AGC
                                            Met Ala Phe Tyr Ser>
                                                TRANSLATION    >

    20          30          40          50          60
     *      *      *      *      *      *      *      *      *
TGC TGC TGG GTC CTC TTG GCA CTC ACC TGG CAC ACC TCT GCC TAC GGG
Cys Cys Trp Val Leu Leu Ala Leu Thr Trp His Thr Ser Ala Tyr Gly>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

        70          80          90          100         110
 *      *      *      *      *      *      *      *      *      *
CCA GAC CAG CGA GCC CAA AAG AAG GGG GAC ATT ATC CTT GGG GGG CTC
Pro Asp Gln Arg Ala Gln Lys Lys Gly Asp Ile Ile Leu Gly Gly Leu>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

          120         130         140         150
    *      *      *      *      *      *      *      *
TTT CCT ATT CAT TTT GGA GTA GCA GCT AAA GAT CAA GAT CTC AAA TCA
Phe Pro Ile His Phe Gly Val Ala Ala Lys Asp Gln Asp Leu Lys Ser>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

160         170         180         190         200
  *      *      *      *      *      *      *      *      *      *
AGG CCG GAG TCT GTG GAA TGT ATC AGG TAT AAT TTC CGT GGG TTT CGC
Arg Pro Glu Ser Val Glu Cys Ile Arg Tyr Asn Phe Arg Gly Phe Arg>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

210         220         230         240         250
  *      *      *      *      *      *      *      *      *      *
TGG TTA CAG GCT ATG ATA TTT GCC ATA GAG GAG ATA AAC AGC AGC CCA
Trp Leu Gln Ala Met Ile Phe Ala Ile Glu Glu Ile Asn Ser Ser Pro>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

    260         270         280         290         300
     *      *      *      *      *      *      *      *      *
GCC CTT CTT CCC AAC TTG ACG CTG GGA TAC AGG ATA TTT GAC ACT TGC
Ala Leu Leu Pro Asn Leu Thr Leu Gly Tyr Arg Ile Phe Asp Thr Cys>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

        310         320         330         340         350
 *      *      *      *      *      *      *      *      *      *
AAC ACC GTT TCT AAG GCC TTG GAA GCC ACC CTG AGT TTT GTT GCT CAA
Asn Thr Val Ser Lys Ala Leu Glu Ala Thr Leu Ser Phe Val Ala Gln>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

          360         370         380         390
    *      *      *      *      *      *      *      *      *
AAC AAA ATT GAT TCT TTG AAC CTT GAT GAG TTC TGC AAC TGC TCA GAG
Asn Lys Ile Asp Ser Leu Asn Leu Asp Glu Phe Cys Asn Cys Ser Glu>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

400         410         420         430         440
  *      *      *      *      *      *      *      *      *      *
CAC ATT CCC TCT ACG ATT GCT GTG GTG GGA GCA ACT GGC TCA GGC GTC
His Ile Pro Ser Thr Ile Ala Val Val Gly Ala Thr Gly Ser Gly Val>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >
```

FIG. 3b.

```
450         460         470         480         490
 *     *     *     *     *     *     *     *     *     *
TCC ACG GCA GTG GCA AAT CTG CTG GGG CTC TTC TAC ATT CCC CAG GTC
Ser Thr Ala Val Ala Asn Leu Leu Gly Leu Phe Tyr Ile Pro Gln Val>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

  500         510         520         530         540
   *     *     *     *     *     *     *     *     *
AGT TAT GCC TCC TCC AGC AGA CTC CTC AGC AAC AAG AAT CAA TTC AAG
Ser Tyr Ala Ser Ser Ser Arg Leu Leu Ser Asn Lys Asn Gln Phe Lys>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

     550         560         570         580         590
 *     *     *     *     *     *     *     *     *     *
TCT TTC CTC CGA ACC ATC CCC AAT GAT GAG CAC CAG GCC ACT GCC ATG
Ser Phe Leu Arg Thr Ile Pro Asn Asp Glu His Gln Ala Thr Ala Met>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

        600         610         620         630
   *     *     *     *     *     *     *     *     *
GCA GAC ATC ATC GAG TAT TTC CGC TGG AAC TGG GTG GGC ACA ATT GCA
Ala Asp Ile Ile Glu Tyr Phe Arg Trp Asn Trp Val Gly Thr Ile Ala>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

640         650         660         670         680
 *     *     *     *     *     *     *     *     *     *
GCT GAT GAC GAC TAT GGG CGG CCG GGG ATT GAG AAA TTC CGA GAG GAA
Ala Asp Asp Asp Tyr Gly Arg Pro Gly Ile Glu Lys Phe Arg Glu Glu>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

690         700         710         720         730
 *     *     *     *     *     *     *     *     *     *
GCT GAG GAA AGG GAT ATC TGC ATC GAC TTC AGT GAA CTC ATC TCC CAG
Ala Glu Glu Arg Asp Ile Cys Ile Asp Phe Ser Glu Leu Ile Ser Gln>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

   740         750         760         770         780
    *     *     *     *     *     *     *     *     *
TAC TCT GAT GAG GAA GAG ATC CAG CAT GTG GTA GAG GTG ATT CAA AAT
Tyr Ser Asp Glu Glu Glu Ile Gln His Val Val Glu Val Ile Gln Asn>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

     790         800         810         820         830
 *     *     *     *     *     *     *     *     *     *
TCC ACG GCC AAA GTC ATC GTG GTT TTC TCC AGT GGC CCA GAT CTT GAG
Ser Thr Ala Lys Val Ile Val Val Phe Ser Ser Gly Pro Asp Leu Glu>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

        840         850         860         870
   *     *     *     *     *     *     *     *     *
CCC CTC ATC AAG GAG ATT GTC CGG CGC AAT ATC ACG GGC AAG ATC TGG
Pro Leu Ile Lys Glu Ile Val Arg Arg Asn Ile Thr Gly Lys Ile Trp>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

880         890         900         910         920
 *     *     *     *     *     *     *     *     *     *
CTG GCC AGC GAG GCC TGG GCC AGC TCC TCC CTG ATC GCC ATG CCT CAG
Leu Ala Ser Glu Ala Trp Ala Ser Ser Ser Leu Ile Ala Met Pro Gln>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

930         940         950         960         970
 *     *     *     *     *     *     *     *     *     *
```

FIG. 3c.

```
TAC TTC CAC GTG GTT GGC GGC ACC ATT GGA TTC GCT CTG AAG GCT GGG
Tyr Phe His Val Val Gly Gly Thr Ile Gly Phe Ala Leu Lys Ala Gly>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

   980       990       1000      1010      1020
     *    *    *    *    *    *    *    *    *

CAG ATC CCA GGC TTC CGG GAA TTC CTG AAG AAG GTC CAT CCC AGG AAG
Gln Ile Pro Gly Phe Arg Glu Phe Leu Lys Lys Val His Pro Arg Lys>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

     1030      1040      1050      1060      1070
  *    *    *    *    *    *    *    *    *    *

TCT GTC CAC AAT GGT TTT GCC AAG GAG TTT TGG GAA GAA ACA TTT AAC
Ser Val His Asn Gly Phe Ala Lys Glu Phe Trp Glu Glu Thr Phe Asn>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

       1080      1090      1100      1110
    *    *    *    *    *    *    *    *    *

TGC CAC CTC CAA GAA GGT GCA AAA GGA CCT TTA CCT GTG GAC ACC TTT
Cys His Leu Gln Glu Gly Ala Lys Gly Pro Leu Pro Val Asp Thr Phe>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

1120       1130      1140      1150      1160
 *    *    *    *    *    *    *    *    *    *

CTG AGA GGT CAC GAA GAA AGT GGC GAC AGG TTT AGC AAC AGC TCG ACA
Leu Arg Gly His Glu Glu Ser Gly Asp Arg Phe Ser Asn Ser Ser Thr>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

  1170      1180      1190      1200      1210
   *    *    *    *    *    *    *    *    *    *

GCC TTC CGA CCC CTC TGT ACA GGG GAT GAG AAC ATC AGC AGT GTC GAG
Ala Phe Arg Pro Leu Cys Thr Gly Asp Glu Asn Ile Ser Ser Val Glu>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

   1220      1230      1240      1250      1260
     *    *    *    *    *    *    *    *    *

ACC CCT TAC ATA GAT TAC ACG CAT TTA CGG ATA TCC TAC AAT GTG TAC
Thr Pro Tyr Ile Asp Tyr Thr His Leu Arg Ile Ser Tyr Asn Val Tyr>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

     1270      1280      1290      1300      1310
  *    *    *    *    *    *    *    *    *    *

TTA GCA GTC TAC TCC ATT GCC CAC GCC TTG CAA GAT ATA TAT ACC TGC
Leu Ala Val Tyr Ser Ile Ala His Ala Leu Gln Asp Ile Tyr Thr Cys>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

       1320      1330      1340      1350
    *    *    *    *    *    *    *    *    *

TTA CCT GGG AGA GGG CTC TTC ACC AAT GGC TCC TGT GCA GAC ATC AAG
Leu Pro Gly Arg Gly Leu Phe Thr Asn Gly Ser Cys Ala Asp Ile Lys>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

1360       1370      1380      1390      1400
 *    *    *    *    *    *    *    *    *    *

AAA GTT GAG GCG TGG CAG GTC CTG AAG CAC CTA CGG CAT CTA AAC TTT
Lys Val Glu Ala Trp Gln Val Leu Lys His Leu Arg His Leu Asn Phe>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

  1410      1420      1430      1440      1450
   *    *    *    *    *    *    *    *    *    *

ACA AAC AAT ATG GGG GAG CAG GTG ACC TTT GAT GAG TGT GGT GAC CTG
Thr Asn Asn Met Gly Glu Gln Val Thr Phe Asp Glu Cys Gly Asp Leu>
```

FIG. 3d

a a a a TRANSLATION OF CAR/R1 [A] a a a a >

1460 1470 1480 1490 1500
* * * * * * * * *
GTG GGG AAC TAT TCC ATC ATC AAC TGG CAC CTC TCC CCA GAG GAT GGC
Val Gly Asn Tyr Ser Ile Ile Asn Trp His Leu Ser Pro Glu Asp Gly>
a a a a TRANSLATION OF CAR/R1 [A] a a a a >

1510 1520 1530 1540 1550
* * * * * * * * * *
TCC ATC GTG TTT AAG GAA GTC GGG TAT TAC AAC GTC TAT GCC AAG AAG
Ser Ile Val Phe Lys Glu Val Gly Tyr Tyr Asn Val Tyr Ala Lys Lys>
a a a a TRANSLATION OF CAR/R1 [A] a a a a >

1560 1570 1580 1590
* * * * * * * * *
GGA GAA AGA CTC TTC ATC AAC GAG GAG AAA ATC CTG TGG AGT GGG TTC
Gly Glu Arg Leu Phe Ile Asn Glu Glu Lys Ile Leu Trp Ser Gly Phe>
a a a a TRANSLATION OF CAR/R1 [A] a a a a >

1600 1610 1620 1630 1640
* * * * * * * * * *
TCC AGG GAG GTG CCC TTC TCC AAC TGC AGC CGA GAC TGC CTG GCA GGG
Ser Arg Glu Val Pro Phe Ser Asn Cys Ser Arg Asp Cys Leu Ala Gly>
a a a a TRANSLATION OF CAR/R1 [A] a a a a >

1650 1660 1670 1680 1690
* * * * * * * * * *
ACC AGG AAA GGG ATC ATT GAG GGG GAG CCC ACC TGC TGC TTT GAG TGT
Thr Arg Lys Gly Ile Ile Glu Gly Glu Pro Thr Cys Cys Phe Glu Cys>
a a a a TRANSLATION OF CAR/R1 [A] a a a a >

1700 1710 1720 1730 1740
* * * * * * * * *
GTG GAG TGT CCT GAT GGG GAG TAT AGT GAT GAG ACA GAT GCC AGT GCC
Val Glu Cys Pro Asp Gly Glu Tyr Ser Asp Glu Thr Asp Ala Ser Ala>
a a a a TRANSLATION OF CAR/R1 [A] a a a a >

1750 1760 1770 1780 1790
* * * * * * * * * *
TGT AAC AAG TGC CCA GAT GAC TTC TGG TCC AAT GAG AAC CAC ACC TCC
Cys Asn Lys Cys Pro Asp Asp Phe Trp Ser Asn Glu Asn His Thr Ser>
a a a a TRANSLATION OF CAR/R1 [A] a a a a >

1800 1810 1820 1830
* * * * * * * * *
TGC GAG CCC ATT CCT GTC CGT TAT CTT GAG TGG AGT GAC ATA GAA TCT
Cys Glu Pro Ile Pro Val Arg Tyr Leu Glu Trp Ser Asp Ile Glu Ser>
a a a a TRANSLATION OF CAR/R1 [A] a a a a >

1840 1850 1860 1870 1880
* * * * * * * * * *
ATC ATA GCC ATC GCC TTT TCT TGC CTG GGC ATC CTC GTG ACG CTG TTT
Ile Ile Ala Ile Ala Phe Ser Cys Leu Gly Ile Leu Val Thr Leu Phe>
a a a a TRANSLATION OF CAR/R1 [A] a a a a >

1890 1900 1910 1920 1930
* * * * * * * * * *
GTC ACC CTC ATC TTC GTT CTG TAC CGG GAC ACA CCC GTG GTC AAA TCC
Val Thr Leu Ile Phe Val Leu Tyr Arg Asp Thr Pro Val Val Lys Ser>
a a a a TRANSLATION OF CAR/R1 [A] a a a a >

FIG. 3e.

```
  1940      1950       1960       1970       1980
    *    *    *    *    *    *    *    *    *
TCC AGT AGG GAG CTC TGC TAT ATC ATT CTG GCT GGT ATT TTC CTC GGC
Ser Ser Arg Glu Leu Cys Tyr Ile Ile Leu Ala Gly Ile Phe Leu Gly>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]    a   a   a   a   >

    1990       2000       2010       2020       2030
 *    *    *    *    *    *    *    *    *    *
TAT GTG TGC CCT TTC ACC CTC ATC GCC AAA CCT ACT ACC ACA TCC TGC
Tyr Val Cys Pro Phe Thr Leu Ile Ala Lys Pro Thr Thr Thr Ser Cys>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]    a   a   a   a   >

      2040       2050       2060       2070
    *    *    *    *    *    *    *    *    *
TAC CTC CAG CGC CTC CTA GTT GGC CTC TCT TCT GCC ATG TGC TAC TCT
Tyr Leu Gln Arg Leu Leu Val Gly Leu Ser Ser Ala Met Cys Tyr Ser>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]    a   a   a   a   >

2080       2090       2100       2110       2120
 *    *    *    *    *    *    *    *    *    *
GCT TTA GTG ACC AAA ACC AAT CGT ATT GCA CGC ATC CTG GCT GGC AGC
Ala Leu Val Thr Lys Thr Asn Arg Ile Ala Arg Ile Leu Ala Gly Ser>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]    a   a   a   a   >

  2130       2140       2150       2160       2170
   *    *    *    *    *    *    *    *    *    *
AAG AAG AAG ATC TGC ACC CGG AAG CCC AGA TTC ATG AGC GCT TGG GCC
Lys Lys Lys Ile Cys Thr Arg Lys Pro Arg Phe Met Ser Ala Trp Ala>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]    a   a   a   a   >

   2180       2190       2200       2210       2220
    *    *    *    *    *    *    *    *    *
CAA GTG ATC ATA GCC TCC ATT CTG ATT AGT GTA CAG CTA ACA CTA GTG
Gln Val Ile Ile Ala Ser Ile Leu Ile Ser Val Gln Leu Thr Leu Val>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]    a   a   a   a   >

     2230       2240       2250       2260       2270
 *    *    *    *    *    *    *    *    *    *
GTG ACC TTG ATC ATC ATG GAG CCT CCC ATG CCC ATT TTG TCC TAC CCG
Val Thr Leu Ile Ile Met Glu Pro Pro Met Pro Ile Leu Ser Tyr Pro>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]    a   a   a   a   >

      2280       2290       2300       2310
    *    *    *    *    *    *    *    *    *
AGT ATC AAG GAA GTC TAC CTT ATC TGC AAT ACC AGC AAC CTG GGT GTA
Ser Ile Lys Glu Val Tyr Leu Ile Cys Asn Thr Ser Asn Leu Gly Val>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]    a   a   a   a   >

2320       2330       2340       2350       2360
 *    *    *    *    *    *    *    *    *    *
GTG GCC CCT GTG GGT TAC AAT GGA CTC CTC ATC ATG AGC TGT ACC TAC
Val Ala Pro Val Gly Tyr Asn Gly Leu Leu Ile Met Ser Cys Thr Tyr>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]    a   a   a   a   >

 2370       2380       2390       2400       2410
   *    *    *    *    *    *    *    *    *    *
TAT GCC TTC AAG ACC CGC AAC GTG CCG GCC AAC TTC AAT GAG GCT AAA
Tyr Ala Phe Lys Thr Arg Asn Val Pro Ala Asn Phe Asn Glu Ala Lys>
  a   a   a   a  TRANSLATION OF CAR/R1 [A]    a   a   a   a   >

   2420       2430       2440       2450       2460
    *    *    *    *    *    *    *    *    *
```

FIG. 3f

```
TAC ATC GCC TTC ACC ATG TAC ACT ACC TGC ATC ATC TGG CTG GCT TTC
Tyr Ile Ala Phe Thr Met Tyr Thr Thr Cys Ile Ile Trp Leu Ala Phe>
   a   a   a   a  TRANSLATION OF CAR/R1 [A]    a   a   a   a   >

      2470        2480        2490        2500        2510
  *     *     *     *     *     *     *     *     *     *
GTT CCC ATT TAC TTT GGG AGC AAC TAC AAG ATC ATC ACT ACC TGC TTC
Val Pro Ile Tyr Phe Gly Ser Asn Tyr Lys Ile Ile Thr Thr Cys Phe>
   a   a   a   a  TRANSLATION OF CAR/R1 [A]    a   a   a   a   >

        2520        2530        2540        2550
    *     *     *     *     *     *     *     *     *
GCG GTG AGC CTC AGT GTG ACG GTG GCC CTG GGG TGC ATG TTT ACT CCG
Ala Val Ser Leu Ser Val Thr Val Ala Leu Gly Cys Met Phe Thr Pro>
   a   a   a   a  TRANSLATION OF CAR/R1 [A]    a   a   a   a   >

2560        2570        2580        2590        2600
  *     *     *     *     *     *     *     *     *     *
AAG ATG TAC ATC ATC ATT GCC AAA CCT GAG AGG AAC GTC CGC AGT GCC
Lys Met Tyr Ile Ile Ile Ala Lys Pro Glu Arg Asn Val Arg Ser Ala>
   a   a   a   a  TRANSLATION OF CAR/R1 [A]    a   a   a   a   >

  2610        2620        2630        2640        2650
    *     *     *     *     *     *     *     *     *     *
TTC ACG ACC TCT GAT GTT GTC CGC ATG CAC GTC GGT GAT GGC AAA CTG
Phe Thr Thr Ser Asp Val Val Arg Met His Val Gly Asp Gly Lys Leu>
   a   a   a   a  TRANSLATION OF CAR/R1 [A]    a   a   a   a   >

    2660        2670        2680        2690        2700
      *     *     *     *     *     *     *     *     *
CCG TGC CGC TCC AAC ACC TTC CTC AAC ATT TTC CGG AGA AAG AAG CCC
Pro Cys Arg Ser Asn Thr Phe Leu Asn Ile Phe Arg Arg Lys Lys Pro>
   a   a   a   a  TRANSLATION OF CAR/R1 [A]    a   a   a   a   >

      2710        2720        2730        2740        2750
  *     *     *     *     *     *     *     *     *     *
GGG GCA GGG AAT GCC AAT TCT AAC GGC AAG TCT GTG TCA TGG TCT GAA
Gly Ala Gly Asn Ala Asn Ser Asn Gly Lys Ser Val Ser Trp Ser Glu>
   a   a   a   a  TRANSLATION OF CAR/R1 [A]    a   a   a   a   >

        2760        2770        2780        2790
    *     *     *     *     *     *     *     *     *
CCA GGT GGA AGA CAG GCG CCC AAG GGA CAG CAC GTG TGG CAG CGC CTC
Pro Gly Gly Arg Gln Ala Pro Lys Gly Gln His Val Trp Gln Arg Leu>
   a   a   a   a  TRANSLATION OF CAR/R1 [A]    a   a   a   a   >

2800        2810        2820        2830        2840
  *     *     *     *     *     *     *     *     *     *
TCT GTG CAC GTG AAG ACC AAC GAG ACG GCC TGT AAC CAA ACA GCC GTA
Ser Val His Val Lys Thr Asn Glu Thr Ala Cys Asn Gln Thr Ala Val>
   a   a   a   a  TRANSLATION OF CAR/R1 [A]    a   a   a   a   >

  2850        2860        2870        2880        2890
    *     *     *     *     *     *     *     *     *     *
ATC AAA CCC CTC ACT AAA AGT TAC CAA GGC TCT GGC AAG AGC CTG ACC
Ile Lys Pro Leu Thr Lys Ser Tyr Gln Gly Ser Gly Lys Ser Leu Thr>
   a   a   a   a  TRANSLATION OF CAR/R1 [A]    a   a   a   a   >

    2900        2910        2920        2930        2940
      *     *     *     *     *     *     *     *     *
TTT TCA GAT GCC AGC ACC AAG ACC CTT TAC AAT GTG GAA GAA GAG GAC
Phe Ser Asp Ala Ser Thr Lys Thr Leu Tyr Asn Val Glu Glu Glu Asp>
```

FIG. 3g.

```
   a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a  >

      2950        2960        2970        2980        2990
 *       *       *       *       *       *       *       *       *       *
AAT ACC CCT TCT GCT CAC TTC AGC CCT CCC AGC AGC CCT TCT ATG GTG
Asn Thr Pro Ser Ala His Phe Ser Pro Pro Ser Ser Pro Ser Met Val>
   a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a  >

        3000        3010        3020        3030
    *       *       *       *       *       *       *       *       *
GTG CAC CGA CGC GGG CCA CCC GTG GCC ACC ACA CCA CCT CTG CCA CCC
Val His Arg Arg Gly Pro Pro Val Ala Thr Thr Pro Pro Leu Pro Pro>
   a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a  >

3040        3050        3060        3070        3080
 *       *       *       *       *       *       *       *       *       *
CAT CTG ACC GCA GAA GAG ACC CCC CTG TTC CTG GCT GAT TCC GTC ATC
His Leu Thr Ala Glu Glu Thr Pro Leu Phe Leu Ala Asp Ser Val Ile>
   a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a  >

  3090        3100        3110        3120        3130
   *       *       *       *       *       *       *       *       *       *
CCC AAG GGC TTG CCT CCT CCT CTC CCG CAG CAG CAG CCA CAG CAG CCG
Pro Lys Gly Leu Pro Pro Pro Leu Pro Gln Gln Gln Pro Gln Gln Pro>
   a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a  >

    3140        3150        3160        3170        3180
     *       *       *       *       *       *       *       *       *
CCC CCT CAG CAG CCC CCG CAG CAG CCC AAG TCC CTG ATG GAC CAG CTG
Pro Pro Gln Gln Pro Pro Gln Gln Pro Lys Ser Leu Met Asp Gln Leu>
   a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a  >

      3190        3200        3210        3220        3230
 *       *       *       *       *       *       *       *       *       *
CAA GGC GTA GTC ACC AAC TTC GGT TCG GGG ATT CCA GAT TTC CAT GCG
Gln Gly Val Val Thr Asn Phe Gly Ser Gly Ile Pro Asp Phe His Ala>
   a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a  >

        3240        3250        3260        3270
    *       *       *       *       *       *       *       *       *
GTG CTG GCA GGC CCG GGG ACA CCA GGA AAC AGC CTG CGC TCT CTG TAC
Val Leu Ala Gly Pro Gly Thr Pro Gly Asn Ser Leu Arg Ser Leu Tyr>
   a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a  >

3280        3290        3300        3310        3320
 *       *       *       *       *       *       *       *       *       *
CCG CCC CCG CCT CCG CCG CAA CAC CTG CAG ATG CTG CCC CTG CAC CTG
Pro Pro Pro Pro Pro Pro Gln His Leu Gln Met Leu Pro Leu His Leu>
   a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a  >

  3330        3340        3350        3360        3370
   *       *       *       *       *       *       *       *       *       *
AGC ACC TTC CAG GAG GAG TCC ATC TCC CCT CCT GGG GAG GAC ATC GAT
Ser Thr Phe Gln Glu Glu Ser Ile Ser Pro Pro Gly Glu Asp Ile Asp>
   a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a  >

    3380        3390        3400        3410        3420
     *       *       *       *       *       *       *       *       *
GAT GAC AGT GAG AGA TTC AAG CTC CTG CAG GAG TTC GTG TAC GAG CGC
Asp Asp Ser Glu Arg Phe Lys Leu Leu Gln Glu Phe Val Tyr Glu Arg>
   a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a  >
```

FIG. 3h.

```
      3430        3440        3450        3460        3470
 *     *     *     *     *     *     *     *     *     *
GAA GGG AAC ACC GAA GAA GAT GAA TTG GAA GAG GAG GAG GAC CTG CCC
Glu Gly Asn Thr Glu Glu Asp Glu Leu Glu Glu Glu Glu Asp Leu Pro>
 a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

        3480        3490        3500        3510
   *     *     *     *     *     *     *     *     *
ACA GCC AGC AAG CTG ACC CCT GAG GAT TCT CCT GCC CTG ACG CCT CCT
Thr Ala Ser Lys Leu Thr Pro Glu Asp Ser Pro Ala Leu Thr Pro Pro>
 a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

3520        3530        3540        3550        3560
 *     *     *     *     *     *     *     *     *     *
TCT CCT TTC CGA GAT TCC GTG GCC TCT GGC AGC TCA GTG CCC AGT TCC
Ser Pro Phe Arg Asp Ser Val Ala Ser Gly Ser Ser Val Pro Ser Ser>
 a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

  3570        3580        3590        3600        3610
   *     *     *     *     *     *     *     *     *     *
CCC GTA TCT GAG TCG GTC CTC TGC ACC CCT CCA AAT GTA ACC TAC GCC
Pro Val Ser Glu Ser Val Leu Cys Thr Pro Pro Asn Val Thr Tyr Ala>
 a   a   a   a  TRANSLATION OF CAR/R1 [A]   a   a   a   a   >

    3620        3630        3640        3650        3660
     *     *     *     *     *     *     *     *     *
TCT GTC ATT CTG AGG GAC TAC AAG CAA AGC TCT TCC ACC CTG TAG
Ser Val Ile Leu Arg Asp Tyr Lys Gln Ser Ser Ser Thr Leu ***>
 a   a   a  TRANSLATION OF CAR/R1 [A]  a   a   a   a   >

      3670      3680      3690      3700      3710      3720
     *    *    *    *    *    *    *    *    *    *    *    *
TGTGTGTGTG TGTGTGGGGG CGGGGGGAGT GCGCATGGAG AAGCCAGAGA TGCCAAGGAG

      3730      3740      3750      3760      3770      3780
     *    *    *    *    *    *    *    *    *    *    *    *
TGTCAACCCT TCCAGAAATG TGTAGAAAGC AGGGTGAGGG ATGGGGATGG AGGACCACGG

      3790      3800      3810      3820      3830      3840
     *    *    *    *    *    *    *    *    *    *    *    *
TCTGCAGGGA AGAAAAAAAA AATGCTGCGG CTGCCTTAAA GAAGGAGAGG GACGATGCCA

      3850      3860      3870      3880      3890      3900
     *    *    *    *    *    *    *    *    *    *    *    *
ACTGAACAGT GGTCCTGGCC AGGATTGTGA CTCTTGAATT ATTCAAAAAC CTTCTCTAGA

      3910      3920      3930      3940      3950      3960
     *    *    *    *    *    *    *    *    *    *    *    *
AAGAAAGGGA ATTATGACAA AGCACAATTC CATATGGTAT GTAACTTTTA TCGAAAAAAA
```

FIG. 4a

```
Sequence Range: -24 to 3195

          -15         -5          6          16          26
        *    *     *    *     *    *     *    *     *    *
GCGGTGGACC GCGTCTTCGC CACA ATG GTC CGG CTC CTC TTG ATT TTC TTC CCA
                           Met Val Arg Leu Leu Leu Ile Phe Phe Pro>
                              a  TRANSLATION OF PRATCH3 [A]    a   >

       36          46          56          66          76
 *    *     *    *     *    *     *    *     *    *
ATG ATC TTT TTG GAG ATG TCC ATT TTG CCC AGG ATG CCT GAC AGA AAA
Met Ile Phe Leu Glu Met Ser Ile Leu Pro Arg Met Pro Asp Arg Lys>
  a   a   a   a  TRANSLATION OF PRATCH3 [A]  a   a   a   a   >

          86          96         106         116         126
   *    *     *    *     *    *     *    *     *    *
GTA TTG CTG GCA GGT GCC TCG TCC CAG CGC TCC GTG GCG AGA ATG GAC
Val Leu Leu Ala Gly Ala Ser Ser Gln Arg Ser Val Ala Arg Met Asp>
  a   a   a   a  TRANSLATION OF PRATCH3 [A]  a   a   a   a   >

            136         146         156         166
     *    *     *    *     *    *     *    *     *
GGA GAT GTC ATC ATC GGA GCC CTC TTC TCA GTC CAT CAC CAG CCT CCA
Gly Asp Val Ile Ile Gly Ala Leu Phe Ser Val His His Gln Pro Pro>
  a   a   a   a  TRANSLATION OF PRATCH3 [A]  a   a   a   a   >

176         186         196         206         216
 *     *    *     *    *     *    *     *    *     *
GCC GAG AAG GTA CCC GAA AGG AAG TGT GGG GAG ATC AGG GAA CAG TAT
Ala Glu Lys Val Pro Glu Arg Lys Cys Gly Glu Ile Arg Glu Gln Tyr>
  a   a   a   a  TRANSLATION OF PRATCH3 [A]  a   a   a   a   >

   226         236         246         256         266
    *    *     *    *     *    *     *    *     *    *
GGT ATC CAG AGG GTG GAG GCC ATG TTC CAC ACG TTG GAT AAG ATT AAC
Gly Ile Gln Arg Val Glu Ala Met Phe His Thr Leu Asp Lys Ile Asn>
  a   a   a   a  TRANSLATION OF PRATCH3 [A]  a   a   a   a   >

       276         286         296         306         316
 *    *     *    *     *    *     *    *     *    *
GCG GAC CCG GTG CTC CTG CCC AAC ATC ACT CTG GGC AGT GAG ATC CGG
Ala Asp Pro Val Leu Leu Pro Asn Ile Thr Leu Gly Ser Glu Ile Arg>
  a   a   a   a  TRANSLATION OF PRATCH3 [A]  a   a   a   a   >

          326         336         346         356         366
   *    *     *    *     *    *     *    *     *    *
GAC TCC TGC TGG CAC TCT TCA GTG GCT CTC GAA CAG AGC ATC GAA TTC
Asp Ser Cys Trp His Ser Ser Val Ala Leu Glu Gln Ser Ile Glu Phe>
  a   a   a   a  TRANSLATION OF PRATCH3 [A]  a   a   a   a   >

             376         386         396         406
     *    *     *    *     *    *     *    *     *
ATC AGA GAC TCC CTG ATT TCC ATC CGA GAT GAG AAG GAT GGG CTG AAC
Ile Arg Asp Ser Leu Ile Ser Ile Arg Asp Glu Lys Asp Gly Leu Asn>
  a   a   a   a  TRANSLATION OF PRATCH3 [A]  a   a   a   a   >

416         426         436         446         456
 *     *    *     *    *     *    *     *    *     *
CGA TGC CTG CCT GAT GGC CAG ACC CTG CCC CCT GGC AGG ACT AAG AAG
Arg Cys Leu Pro Asp Gly Gln Thr Leu Pro Pro Gly Arg Thr Lys Lys>
  a   a   a   a  TRANSLATION OF PRATCH3 [A]  a   a   a   a   >
```

FIG. 4b

```
 466         476         486         496         506
   *     *     *     *     *     *     *     *     *
CCT ATT GCT GGA GTG ATC GGC CCT GGC TCC AGC TCT GTG GCC ATT CAA
Pro Ile Ala Gly Val Ile Gly Pro Gly Ser Ser Ser Val Ala Ile Gln>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]  a   a   a   a  >

    516         526         536         546         556
*     *     *     *     *     *     *     *     *     *
GTC CAG AAT CTT CTC CAG CTG TTC GAC ATC CCA CAG ATC GCC TAT TCT
Val Gln Asn Leu Leu Gln Leu Phe Asp Ile Pro Gln Ile Ala Tyr Ser>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]  a   a   a   a  >

       566         576         586         596         606
  *     *     *     *     *     *     *     *     *     *
GCC ACA AGC ATA GAC CTG AGT GAC AAA ACT TTG TAC AAA TAC TTC CTG
Ala Thr Ser Ile Asp Leu Ser Asp Lys Thr Leu Tyr Lys Tyr Phe Leu>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]  a   a   a   a  >

          616         626         636         646
    *     *     *     *     *     *     *     *     *
AGG GTG GTC CCT TCT GAC ACT TTG CAG GCA AGG GCG ATG CTC GAC ATA
Arg Val Val Pro Ser Asp Thr Leu Gln Ala Arg Ala Met Leu Asp Ile>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]  a   a   a   a  >

656         666         676         686         696
 *     *     *     *     *     *     *     *     *     *
GTC AAG CGT TAC AAC TGG ACC TAT GTC TCA GCA GTC CAC ACA GAA GGG
Val Lys Arg Tyr Asn Trp Thr Tyr Val Ser Ala Val His Thr Glu Gly>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]  a   a   a   a  >

  706         716         726         736         746
   *     *     *     *     *     *     *     *     *
AAT TAC GGC GAG AGT GGA ATG GAT GCT TTC AAA GAA CTG GCT GCC CAG
Asn Tyr Gly Glu Ser Gly Met Asp Ala Phe Lys Glu Leu Ala Ala Gln>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]  a   a   a   a  >

    756         766         776         786         796
*     *     *     *     *     *     *     *     *     *
GAA GGC CTC TGC ATC GCA CAC TCG GAC AAA ATC TAC AGC AAT GCT GGC
Glu Gly Leu Cys Ile Ala His Ser Asp Lys Ile Tyr Ser Asn Ala Gly>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]  a   a   a   a  >

       806         816         826         836         846
  *     *     *     *     *     *     *     *     *     *
GAG AAG AGC TTT GAC CGG CTC CTG CGT AAA CTC CGG GAG CGG CTT CCC
Glu Lys Ser Phe Asp Arg Leu Leu Arg Lys Leu Arg Glu Arg Leu Pro>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]  a   a   a   a  >

          856         866         876         886
    *     *     *     *     *     *     *     *     *
AAG GCC AGG GTT GTG GTC TGC TTC TGC GAG GGC ATG ACA GTG CGG GGC
Lys Ala Arg Val Val Val Cys Phe Cys Glu Gly Met Thr Val Arg Gly>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]  a   a   a   a  >

896         906         916         926         936
 *     *     *     *     *     *     *     *     *     *
TTA CTG AGT GCC ATG CGC CGC CTG GGC GTC GTG GGC GAG TTC TCA CTC
Leu Leu Ser Ala Met Arg Arg Leu Gly Val Val Gly Glu Phe Ser Leu>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]  a   a   a   a  >

  946         956         966         976         986
   *     *     *     *     *     *     *     *     *
```

FIG. 4c.

```
ATT GGA AGT GAT GGA TGG GCA GAC AGA GAT GAA GTC ATC GAA GGC TAT
Ile Gly Ser Asp Gly Trp Ala Asp Arg Asp Glu Val Ile Glu Gly Tyr>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a   >

     996         1006         1016         1026         1036
 *     *     *     *     *     *     *     *     *     *
GAG GTG GAA GCC AAC GGA GGG ATC ACA ATA AAG CTT CAG TCT CCA GAG
Glu Val Glu Ala Asn Gly Gly Ile Thr Ile Lys Leu Gln Ser Pro Glu>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a   >

       1046         1056         1066         1076         1086
   *     *     *     *     *     *     *     *     *     *
GTC AGG TCA TTT GAT GAC TAC TTC CTG AAG CTG AGG CTG GAC ACC AAC
Val Arg Ser Phe Asp Asp Tyr Phe Leu Lys Leu Arg Leu Asp Thr Asn>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a   >

          1096         1106         1116         1126
     *     *     *     *     *     *     *     *     *
ACA AGG AAT CCT TGG TTC CCT GAG TTC TGG CAA CAT CGC TTC CAG TGT
Thr Arg Asn Pro Trp Phe Pro Glu Phe Trp Gln His Arg Phe Gln Cys>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a   >

1136         1146         1156         1166         1176
  *     *     *     *     *     *     *     *     *     *
CGC CTA CCT GGA CAC CTC TTG GAA AAC CCC AAC TTT AAG AAA GTG TGC
Arg Leu Pro Gly His Leu Leu Glu Asn Pro Asn Phe Lys Lys Val Cys>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a   >

  1186         1196         1206         1216         1226
     *     *     *     *     *     *     *     *     *     *
ACA GGA AAT GAA AGC TTG GAA GAA AAC TAT GTC CAG GAC AGC AAA ATG
Thr Gly Asn Glu Ser Leu Glu Glu Asn Tyr Val Gln Asp Ser Lys Met>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a   >

    1236         1246         1256         1266         1276
 *     *     *     *     *     *     *     *     *     *
GGA TTT GTC ATC AAT GCC ATC TAT GCC ATG GCA CAT GGG CTG CAG AAC
Gly Phe Val Ile Asn Ala Ile Tyr Ala Met Ala His Gly Leu Gln Asn>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a   >

       1286         1296         1306         1316         1326
   *     *     *     *     *     *     *     *     *     *
ATG CAC CAT GCT CTG TGT CCC GGC CAT GTG GGC CTG TGT GAT GCT ATG
Met His His Ala Leu Cys Pro Gly His Val Gly Leu Cys Asp Ala Met>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a   >

          1336         1346         1356         1366
     *     *     *     *     *     *     *     *     *
AAA CCC ATT GAT GGC AGG AAG CTC CTG GAT TTC CTC ATC AAA TCC TCT
Lys Pro Ile Asp Gly Arg Lys Leu Leu Asp Phe Leu Ile Lys Ser Ser>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a   >

1376         1386         1396         1406         1416
  *     *     *     *     *     *     *     *     *     *
TTT GTC GGA GTG TCT GGA GAG GAG GTG TGG TTC GAT GAG AAG GGG GAT
Phe Val Gly Val Ser Gly Glu Glu Val Trp Ph  Asp Glu Lys Gly Asp>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a   >

  1426         1436         1446         1456         1466
     *     *     *     *     *     *     *     *     *
GCT CCC GGA AGG TAT GAC ATT ATG AAT CTG CAG TAC ACA GAA GCT AAT
Ala Pro Gly Arg Tyr Asp Ile Met Asn Leu Gln Tyr Thr Glu Ala Asn>
```

FIG. 4d.

```
    a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a  >

   1476              1486            1496            1506             1516
 *       *       *       *       *       *       *       *       *       *
CGC TAT GAC TAT GTC CAC GTG GGG ACC TGG CAT GAA GGA GTG CTG AAT
Arg Tyr Asp Tyr Val His Val Gly Thr Trp His Glu Gly Val Leu Asn>
    a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a  >

       1526            1536            1546            1556            1566
   *       *       *       *       *       *       *       *       *       *
ATT GAT GAT TAC AAA ATC CAG ATG AAC AAA AGC GGA ATG GTA CGA TCT
Ile Asp Asp Tyr Lys Ile Gln Met Asn Lys Ser Gly Met Val Arg Ser>
    a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a  >

          1576            1586            1596            1606
     *       *       *       *       *       *       *       *       *
GTG TGC AGT GAG CCT TGC TTA AAG GGT CAG ATT AAG GTC ATA CGG AAA
Val Cys Ser Glu Pro Cys Leu Lys Gly Gln Ile Lys Val Ile Arg Lys>
    a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a  >

1616            1626            1636            1646            1656
  *       *       *       *       *       *       *       *       *       *
GGA GAA GTG AGC TGC TGC TGG ATC TGC ACG GCC TGC AAA GAG AAT GAG
Gly Glu Val Ser Cys Cys Trp Ile Cys Thr Ala Cys Lys Glu Asn Glu>
    a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a  >

   1666            1676            1686            1696            1706
     *       *       *       *       *       *       *       *       *
TTT GTG CAG GAC GAG TTC ACC TGC AGA GCC TGT GAC CTG GGG TGG TGG
Phe Val Gln Asp Glu Phe Thr Cys Arg Ala Cys Asp Leu Gly Trp Trp>
    a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a  >

   1716              1726            1736            1746             1756
 *       *       *       *       *       *       *       *       *       *
CCC AAC GCA GAG CTC ACA GGC TGT GAG CCC ATT CCT GTC CGT TAT CTT
Pro Asn Ala Glu Leu Thr Gly Cys Glu Pro Ile Pro Val Arg Tyr Leu>
    a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a  >

       1766            1776            1786            1796            1806
   *       *       *       *       *       *       *       *       *       *
GAG TGG AGT GAC ATA GAA TCT ATC ATA GCC ATC GCC TTT TCT TGC CTG
Glu Trp Ser Asp Ile Glu Ser Ile Ile Ala Ile Ala Phe Ser Cys Leu>
    a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a  >

          1816            1826            1836            1846
     *       *       *       *       *       *       *       *       *
GGC ATC CTC GTG ACG CTG TTT GTC ACC CTC ATC TTC GTT CTG TAC CGG
Gly Ile Leu Val Thr Leu Phe Val Thr Leu Ile Phe Val Leu Tyr Arg>
    a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a  >

1856            1866            1876            1886            1896
  *       *       *       *       *       *       *       *       *       *
GAC ACA CCC GTG GTC AAA TCC TCC AGT AGG GAG CTC TGC TAT ATC ATT
Asp Thr Pro Val Val Lys Ser Ser Ser Arg Glu Leu Cys Tyr Ile Ile>
    a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a  >

   1906            1916            1926            1936            1946
     *       *       *       *       *       *       *       *       *
CTG GCT GGT ATT TTC CTC GGC TAT GTG TGC CCT TTC ACC CTC ATC GCC
Leu Ala Gly Ile Phe Leu Gly Tyr Val Cys Pro Phe Thr Leu Ile Ala>
    a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a  >
```

FIG. 4e.

```
 1956      1966      1976      1986      1996
*    *    *    *    *    *    *    *    *    *
AAA CCT ACT ACC ACA TCC TGC TAC CTC CAG CGC CTC CTA GTT GGC CTC
Lys Pro Thr Thr Thr Ser Cys Tyr Leu Gln Arg Leu Leu Val Gly Leu>
  a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a   >

     2006      2016      2026      2036      2046
   *    *    *    *    *    *    *    *    *    *
TCT TCT GCC ATG TGC TAC TCT GCT TTA GTG ACC AAA ACC AAT CGT ATT
Ser Ser Ala Met Cys Tyr Ser Ala Leu Val Thr Lys Thr Asn Arg Ile>
  a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a   >

        2056      2066      2076      2086
      *    *    *    *    *    *    *    *    *
GCA CGC ATC CTG GCT GGC AGC AAG AAG AAG ATC TGC ACC CGG AAG CCC
Ala Arg Ile Leu Ala Gly Ser Lys Lys Lys Ile Cys Thr Arg Lys Pro>
  a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a   >

2096      2106      2116      2126      2136
 *    *    *    *    *    *    *    *    *    *
AGA TTC ATG AGC GCT TGG GCC CAA GTG ATC ATA GCC TCC ATT CTG ATT
Arg Phe Met Ser Ala Trp Ala Gln Val Ile Ile Ala Ser Ile Leu Ile>
  a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a   >

  2146      2156      2166      2176      2186
     *    *    *    *    *    *    *    *    *
AGT GTA CAG CTA ACA CTA GTG GTG ACC TTG ATC ATC ATG GAG CCT CCC
Ser Val Gln Leu Thr Leu Val Val Thr Leu Ile Ile Met Glu Pro Pro>
  a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a   >

    2196      2206      2216      2226      2236
*    *    *    *    *    *    *    *    *    *
ATG CCC ATT TTG TCC TAC CCG AGT ATC AAG GAA GTC TAC CTT ATC TGC
Met Pro Ile Leu Ser Tyr Pro Ser Ile Lys Glu Val Tyr Leu Ile Cys>
  a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a   >

     2246      2256      2266      2276      2286
   *    *    *    *    *    *    *    *    *    *
AAT ACC AGC AAC CTG GGT GTG GTG GCC CCT TTG GGC TAC AAT GGA CTC
Asn Thr Ser Asn Leu Gly Val Val Ala Pro Leu Gly Tyr Asn Gly Leu>
  a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a   >

        2296      2306      2316      2326
      *    *    *    *    *    *    *    *    *
CTC ATC ATG AGC TGT ACC TAC TAT GCC TTC AAG ACC CGC AAC GTG CCC
Leu Ile Met Ser Cys Thr Tyr Tyr Ala Phe Lys Thr Arg Asn Val Pro>
  a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a   >

2336      2346      2356      2366      2376
 *    *    *    *    *    *    *    *    *    *
GCC AAC TTC AAC GAG GCC AAA TAT ATC GCG TTC ACC ATG TAC ACC ACC
Ala Asn Phe Asn Glu Ala Lys Tyr Ile Ala Phe Thr Met Tyr Thr Thr>
  a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a   >

  2386      2396      2406      2416      2426
     *    *    *    *    *    *    *    *    *
TGT ATC ATC TGG CTA GCT TTT GTG CCC ATT TAC TTT GGG AGC AAC TAC
Cys Ile Ile Trp Leu Ala Phe Val Pro Ile Tyr Phe Gly Ser Asn Tyr>
  a   a   a   a  TRANSLATION OF PRATCH3 [A]   a   a   a   a   >

    2436      2446      2456      2466      2476
*    *    *    *    *    *    *    *    *    *
```

FIG. 4f

```
AAG ATC ATC ACA ACT TGC TTT GCA GTG AGT CTC AGT GTA ACA GTG GCT
Lys Ile Ile Thr Thr Cys Phe Ala Val Ser Leu Ser Val Thr Val Ala>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]  a   a   a   a   >

       2486            2496            2506            2516            2526
   *        *        *        *        *        *        *        *        *        *
CTG GGG TGC ATG TTC ACT CCC AAG ATG TAC ATC ATT ATT GCC AAG CCT
Leu Gly Cys Met Phe Thr Pro Lys Met Tyr Ile Ile Ile Ala Lys Pro>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]  a   a   a   a   >

           2536            2546            2556            2566
       *        *        *        *        *        *        *        *        *
GAG AGG AAT ACC ATC GAG GAG GTG CGT TGC AGC ACC GCA GCT CAC GCT
Glu Arg Asn Thr Ile Glu Glu Val Arg Cys Ser Thr Ala Ala His Ala>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]  a   a   a   a   >

2576            2586            2596            2606            2616
   *        *        *        *        *        *        *        *        *        *
TTC AAG GTG GCT GCC CGG GCC ACG CTG CGC CGC AGC AAC GTC TCC CGC
Phe Lys Val Ala Ala Arg Ala Thr Leu Arg Arg Ser Asn Val Ser Arg>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]  a   a   a   a   >

 2626            2636            2646            2656            2666
       *        *        *        *        *        *        *        *
AAG CGG TCC AGC AGC CTT GGA GGC TCC ACG GGA TCC ACC CCC TCC TCC
Lys Arg Ser Ser Ser Leu Gly Gly Ser Thr Gly Ser Thr Pro Ser Ser>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]  a   a   a   a   >

    2676            2686            2696            2706            2716
*        *        *        *        *        *        *        *        *        *
TCC ATC AGC AGC AAG AGC AAC AGC GAA GAC CCA TTC CCA CAG CCC GAG
Ser Ile Ser Ser Lys Ser Asn Ser Glu Asp Pro Phe Pro Gln Pro Glu>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]  a   a   a   a   >

       2726            2736            2746            2756            2766
   *        *        *        *        *        *        *        *        *        *
AGG CAG AAG CAG CAG CAG CCG CTG GCC CTA ACC CAG CAA GAG CAG CAG
Arg Gln Lys Gln Gln Gln Pro Leu Ala Leu Thr Gln Gln Glu Gln Gln>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]  a   a   a   a   >

           2776            2786            2796            2806
       *        *        *        *        *        *        *        *        *
CAG CAG CCC CTG ACC CTC CCA CAG CAG CAA CGA TCT CAG CAG CAG CCC
Gln Gln Pro Leu Thr Leu Pro Gln Gln Gln Arg Ser Gln Gln Gln Pro>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]  a   a   a   a   >

2816            2826            2836            2846            2856
   *        *        *        *        *        *        *        *        *        *
AGA TGC AAG CAG AAG GTC ATC TTT GGC AGC GGC ACG GTC ACC TTC TCA
Arg Cys Lys Gln Lys Val Ile Phe Gly Ser Gly Thr Val Thr Phe Ser>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]  a   a   a   a   >

 2866            2876            2886            2896            2906
       *        *        *        *        *        *        *        *        *
CTG AGC TTT GAT GAG CCT CAG AAG AAC GCC ATG GCC CAC GGG AAT TCT
Leu Ser Phe Asp Glu Pro Gln Lys Asn Ala Met Ala His Gly Asn Ser>
   a   a   a   a  TRANSLATION OF PRATCH3 [A]  a   a   a   a   >

    2916            2926            2936            2946            2956
*        *        *        *        *        *        *        *        *        *
ACG CAC CAG AAC TCC CTG GAG GCC CAG AAA AGC AGC GAT ACG CTG ACC
Thr His Gln Asn Ser Leu Glu Ala Gln Lys Ser Ser Asp Thr Leu Thr>
```

FIG. 4g

```
    a    a    a    a   TRANSLATION OF PRATCH3 [A]    a    a    a    a   >

        2966          2976           2986          2996          3006
   *       *       *       *       *       *       *       *       *
CGA CAC CAG CCA TTA CTC CCG CTG CAG TGC GGG GAA ACG GAC TTA GAT
Arg His Gln Pro Leu Leu Pro Leu Gln Cys Gly Glu Thr Asp Leu Asp>
    a    a    a    a   TRANSLATION OF PRATCH3 [A]    a    a    a    a   >

           3016          3026          3036          3046
     *       *       *       *       *       *       *       *       *
CTG ACC GTC CAG GAA ACA GGT CTG CAA GGA CCT GTG GGT GGA GAC CAG
Leu Thr Val Gln Glu Thr Gly Leu Gln Gly Pro Val Gly Gly Asp Gln>
    a    a    a    a   TRANSLATION OF PRATCH3 [A]    a    a    a    a   >

3056          3066          3076          3086          3096
  *       *       *       *       *       *       *       *       *       *
CGG CCA GAG GTG GAG GAC CCT GAA GAG TTG TCC CCA GCA CTT GTA GTG
Arg Pro Glu Val Glu Asp Pro Glu Glu Leu Ser Pro Ala Leu Val Val>
    a    a    a    a   TRANSLATION OF PRATCH3 [A]    a    a    a    a   >

  3106          3116          3126          3136          3146
     *       *       *       *       *       *       *       *       *
TCC AGT TCA CAG AGC TTT GTC ATC AGT GGT GGA GGC AGC ACT GTT ACA
Ser Ser Ser Gln Ser Phe Val Ile Ser Gly Gly Gly Ser Thr Val Thr>
    a    a    a    a   TRANSLATION OF PRATCH3 [A]    a    a    a    a   >

    3156          3166          3176          3186
*       *       *       *       *       *       *       *       *
GAA AAC GTA GTG AAT TCA TAAAATGG AAGGAGAAGA CTGGGCTAG
Glu Asn Val Val Asn Ser>
    TRANSLATION OF P    >
```

FIG. 5a

Sequence Range: -24 to 3195

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      -15       -5        6        16        26
       *    *    *    *    *    *    *    *    *    *
GCGGTGGACC GCGTCTTCGC CACA ATG GTC CGG CTC CTC TTG ATT TTC TTC CCA
                          Met Val Arg Leu Leu Leu Ile Phe Phe Pro>
                            a   TRANSLATION OF PHCH4 [A]    a   >

     36        46        56        66        76
 *    *    *    *    *    *    *    *    *    *
ATG ATC TTT TTG GAG ATG TCC ATT TTG CCC AGG ATG CCT GAC AGA AAA
Met Ile Phe Leu Glu Met Ser Ile Leu Pro Arg Met Pro Asp Arg Lys>
  a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

       86        96        106       116       126
  *    *    *    *    *    *    *    *    *    *
GTA TTG CTG GCA GGT GCC TCG TCC CAG CGC TCC GTG GCG AGA ATG GAC
Val Leu Leu Ala Gly Ala Ser Ser Gln Arg Ser Val Ala Arg Met Asp>
  a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

         136       146       156       166
    *    *    *    *    *    *    *    *    *
GGA GAT GTC ATC ATC GGA GCC CTC TTC TCA GTC CAT CAC CAG CCT CCA
Gly Asp Val Ile Ile Gly Ala Leu Phe Ser Val His His Gln Pro Pro>
  a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

176       186       196       206       216
 *    *    *    *    *    *    *    *    *    *
GCC GAG AAG GTA CCC GAA AGG AAG TGT GGG GAG ATC AGG GAA CAG TAT
Ala Glu Lys Val Pro Glu Arg Lys Cys Gly Glu Ile Arg Glu Gln Tyr>
  a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

 226       236       246       256       266
    *    *    *    *    *    *    *    *    *
GGT ATC CAG AGG GTG GAG GCC ATG TTC CAC ACG TTG GAT AAG ATT AAC
Gly Ile Gln Arg Val Glu Ala Met Phe His Thr Leu Asp Lys Ile Asn>
  a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

   276       286       296       306       316
 *    *    *    *    *    *    *    *    *    *
GCG GAC CCG GTG CTC CTG CCC AAC ATC ACT CTG GGC AGT GAG ATC CGG
Ala Asp Pro Val Leu Leu Pro Asn Ile Thr Leu Gly Ser Glu Ile Arg>
  a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

       326       336       346       356       366
  *    *    *    *    *    *    *    *    *    *
GAC TCC TGC TGG CAC TCT TCA GTG GCT CTC GAA CAG AGC ATC GAA TTC
Asp Ser Cys Trp His Ser Ser Val Ala Leu Glu Gln Ser Ile Glu Phe>
  a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

         376       386       396       406
    *    *    *    *    *    *    *    *    *
ATC AGA GAC TCC CTG ATT TCC ATC CGA GAT GAG AAG GAT GGG MTS AAC
Ile Arg Asp Ser Leu Ile Ser Ile Arg Asp Glu Lys Asp Gly Xxx Asn>
  a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

416       426       436       446       456
 *    *    *    *    *    *    *    *    *    *
CGA TGC CTG CCT GAT GGC CAG WCC CTG CCC CCT GGC AGG ACT AAG AAG
Arg Cys Leu Pro Asp Gly Gln Xxx Leu Pro Pro Gly Arg Thr Lys Lys>
  a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >
```

FIG. 5b.

```
   466                 476                 486                 496                 506
    *         *         *         *         *         *         *         *         *
CCT ATT GCT GGA GTG ATC GGC CCT GGC TCC AGC TCT GTG GCC ATT CAA
Pro Ile Ala Gly Val Ile Gly Pro Gly Ser Ser Ser Val Ala Ile Gln>
   a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

       516                 526                 536                 546                 556
*         *         *         *         *         *         *         *         *
GTC CAG AAT CTT CTC CAG CTG TTC GAC ATC CCA CAG ATC GCC TAT TCT
Val Gln Asn Leu Leu Gln Leu Phe Asp Ile Pro Gln Ile Ala Tyr Ser>
   a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

           566                 576                 586                 596                 606
   *         *         *         *         *         *         *         *         *
GCC ACA AGC ATA GAC CTG AGT GAC AAA ACT TTG TAC AAA TAC TTC CTG
Ala Thr Ser Ile Asp Leu Ser Asp Lys Thr Leu Tyr Lys Tyr Phe Leu>
   a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

               616                 626                 636                 646
      *         *         *         *         *         *         *         *
AGG GTT GTC CCT TCT GAC ACT TTG CAG GCA AGG GCC ATG CTT GAC ATA
Arg Val Val Pro Ser Asp Thr Leu Gln Ala Arg Ala Met Leu Asp Ile>
   a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

656                 666                 676                 686                 696
  *         *         *         *         *         *         *         *         *         *
GTC AAA CGT TAC AAT TGG ACC TAT GTC TCT GCA GTC CAC ACG GAA GGG
Val Lys Arg Tyr Asn Trp Thr Tyr Val Ser Ala Val His Thr Glu Gly>
   a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

   706                 716                 726                 736                 746
    *         *         *         *         *         *         *         *         *
AAT TAT GGG GAG AGC GGA ATG GAC GCT TTC AAA GAG CTG GCT GCC CAG
Asn Tyr Gly Glu Ser Gly Met Asp Ala Phe Lys Glu Leu Ala Ala Gln>
   a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

       756                 766                 776                 786                 796
*         *         *         *         *         *         *         *         *
GAA GGC CTC TGT ATC GCC CAT TCT GAC AAA ATC TAC AGC AAC GCT GGG
Glu Gly Leu Cys Ile Ala His Ser Asp Lys Ile Tyr Ser Asn Ala Gly>
   a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

           806                 816                 826                 836                 846
   *         *         *         *         *         *         *         *         *
GAG AAG AGC TTT GAC CGA CTC TTG CGC AAA CTC CGA GAG AGG CTT CCC
Glu Lys Ser Phe Asp Arg Leu Leu Arg Lys Leu Arg Glu Arg Leu Pro>
   a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

               856                 866                 876                 886
      *         *         *         *         *         *         *         *
AAG GCT AGA GTG GTG GTC TGC TTC TGT GAA GGC ATG ACA GTG CGA GGA
Lys Ala Arg Val Val Val Cys Phe Cys Glu Gly Met Thr Val Arg Gly>
   a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

896                 906                 916                 926                 936
  *         *         *         *         *         *         *         *         *         *
CTC CTG AGC GCC ATG CGG CGC CTT GGC GTC GTG GGC GAG TTC TCA CTC
Leu Leu Ser Ala Met Arg Arg Leu Gly Val Val Gly Glu Phe Ser Leu>
   a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

   946                 956                 966                 976                 986
    *         *         *         *         *         *         *         *         *
```

FIG. 5c.

```
ATT GGA AGT GAT GGA TGG GCA GAC AGA GAT GAA GTC ATT GAA GGT TAT
Ile Gly Ser Asp Gly Trp Ala Asp Arg Asp Glu Val Ile Glu Gly Tyr>
   a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

     996        1006        1016        1026        1036
*    *     *     *     *     *     *     *     *     *
GAG GTG GAA GCC AAC GGG GGA ATC ACG ATA AAG CTG CAG TCT CCA GAG
Glu Val Glu Ala Asn Gly Gly Ile Thr Ile Lys Leu Gln Ser Pro Glu>
   a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

       1046        1056        1066        1076        1086
  *     *     *     *     *     *     *     *     *     *
GTC AGG TCA TTT GAT GAT TAT TTC CTG AAA CTG AGG CTG GAC ACT AAC
Val Arg Ser Phe Asp Asp Tyr Phe Leu Lys Leu Arg Leu Asp Thr Asn>
   a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

          1096        1106        1116        1126
    *     *     *     *     *     *     *     *     *
ACG AGG AAT CCC TGG TTC CCT GAG TTC TGG CAA CAT CGG TTC CAG TGC
Thr Arg Asn Pro Trp Phe Pro Glu Phe Trp Gln His Arg Phe Gln Cys>
   a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

1136        1146        1156        1166        1176
 *     *     *     *     *     *     *     *     *     *
CGC CTT CCA GGA CAC CTT CTG GAA AAT CCC AAC TTT AAA CGA ATC TGC
Arg Leu Pro Gly His Leu Leu Glu Asn Pro Asn Phe Lys Arg Ile Cys>
   a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

  1186        1196        1206        1216        1226
    *     *     *     *     *     *     *     *     *
ACA GGC AAT GAA AGC TTA GAA GAA AAC TAT GTC CAG GAC AGT AAG ATG
Thr Gly Asn Glu Ser Leu Glu Glu Asn Tyr Val Gln Asp Ser Lys Met>
   a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

     1236        1246        1256        1266        1276
*    *     *     *     *     *     *     *     *     *
GGG TTT GTC ATC AAT GCC ATC TAT GCC ATG GCA CAT GGG CTG CAG AAC
Gly Phe Val Ile Asn Ala Ile Tyr Ala Met Ala His Gly Leu Gln Asn>
   a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

       1286        1296        1306        1316        1326
  *     *     *     *     *     *     *     *     *     *
ATG CAC CAT GCC CTC TGC CCT GGC CAC GTG GGC CTC TGC GAT GCC ATG
Met His His Ala Leu Cys Pro Gly His Val Gly Leu Cys Asp Ala Met>
   a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

          1336        1346        1356        1366
    *     *     *     *     *     *     *     *     *
AAG CCC ATC GAC GGC AGC AAG CTG CTG GAC TTC CTC ATC AAG TCC TCA
Lys Pro Ile Asp Gly Ser Lys Leu Leu Asp Phe Leu Ile Lys Ser Ser>
   a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

1376        1386        1396        1406        1416
 *     *     *     *     *     *     *     *     *     *
TTC ATT GGA GTA TCT GGA GAG GAG GTG TGG TTT GAT GAG AAA GGA GAC
Phe Ile Gly Val Ser Gly Glu Glu Val Trp Phe Asp Glu Lys Gly Asp>
   a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

  1426        1436        1446        1456        1466
    *     *     *     *     *     *     *     *     *
GCT CCT GGA AGG TAT GAT ATC ATG AAT CTG CAG TAC ACT GAA GCT AAT
Ala Pro Gly Arg Tyr Asp Ile Met Asn Leu Gln Tyr Thr Glu Ala Asn>
```

FIG. 5d

```
 a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

    1476       1486       1496       1506       1516
 *   *   *   *   *   *   *   *   *   *   *
CGC TAT GAC TAT GTG CAC GTT GGA ACC TGG CAT GAA GGA GTG CTG AAC
Arg Tyr Asp Tyr Val His Val Gly Thr Trp His Glu Gly Val Leu Asn>
 a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

    1526       1536       1546       1556       1566
 *   *   *   *   *   *   *   *   *   *   *
ATT GAT GAT TAC AAA ATC CAG ATG AAC AAG AGT GGA GTG GTG CGG TCT
Ile Asp Asp Tyr Lys Ile Gln Met Asn Lys Ser Gly Val Val Arg Ser>
 a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

       1576       1586       1596       1606
 *   *   *   *   *   *   *   *   *
GTG TGC AGT GAG CCT TGC TTA AAG GGC CAG ATT AAG GTT ATA CGG AAA
Val Cys Ser Glu Pro Cys Leu Lys Gly Gln Ile Lys Val Ile Arg Lys>
 a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

1616       1626       1636       1646       1656
 *   *   *   *   *   *   *   *   *   *
GGA GAA GTG AGC TGC TGC TGG ATT TGC ACG GCC TGC AAA GAG AAT GAA
Gly Glu Val Ser Cys Cys Trp Ile Cys Thr Ala Cys Lys Glu Asn Glu>
 a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

  1666       1676       1686       1696       1706
 *   *   *   *   *   *   *   *   *
TAT GTG CAA GAT GAG TTC ACC TGC AAA GCT TGT GAC TTG GGA TGG TGG
Tyr Val Gln Asp Glu Phe Thr Cys Lys Ala Cys Asp Leu Gly Trp Trp>
 a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

  1716       1726       1736       1746       1756
 *   *   *   *   *   *   *   *   *   *   *
CCC AAT GCA GAT CTA ACA GGC TGT GAG CCC ATT CCT GTG CGC TAT CTT
Pro Asn Ala Asp Leu Thr Gly Cys Glu Pro Ile Pro Val Arg Tyr Leu>
 a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

    1766       1776       1786       1796       1806
 *   *   *   *   *   *   *   *   *   *   *
GAG TGG AGC AAC ATC GAA CCC ATT ATA GCC ATC GCC TTT TCA TGC CTG
Glu Trp Ser Asn Ile Glu Pro Ile Ile Ala Ile Ala Phe Ser Cys Leu>
 a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

       1816       1826       1836       1846
 *   *   *   *   *   *   *   *   *
GGA ATC CTT GTT ACC TTG TTT GTC ACC CTA ATC TTT GTA CTG TAC CGG
Gly Ile Leu Val Thr Leu Phe Val Thr Leu Ile Phe Val Leu Tyr Arg>
 a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

1856       1866       1876       1886       1896
 *   *   *   *   *   *   *   *   *   *
GAC ACA CCA GTG GTC AAA TCC TCC AGT CGG GAG CTC TGC TAC ATC ATC
Asp Thr Pro Val Val Lys Ser Ser Ser Arg Glu Leu Cys Tyr Ile Ile>
 a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

  1906       1916       1926       1936       1946
 *   *   *   *   *   *   *   *   *
CTA GCT GGC ATC TTC CTT GGT TAT GTG TGC CCA TTC ACT CTC ATT GCC
Leu Ala Gly Ile Phe Leu Gly Tyr Val Cys Pro Phe Thr Leu Ile Ala>
 a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >
```

FIG. 5e.

```
     1956            1966           1976           1986           1996
       *       *       *       *       *       *       *       *       *       *
AAA CCT ACT ACC ACC TCC TGC TAC CTC CAG CGC CTC TTG GTT GGC CTC
Lys Pro Thr Thr Thr Ser Cys Tyr Leu Gln Arg Leu Leu Val Gly Leu>
  a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

        2006           2016           2026           2036           2046
       *       *       *       *       *       *       *       *       *       *
TCC TCT GCG ATG TGC TAC TCT GCT TTA GTG ACT AAA ACC AAT CGT ATT
Ser Ser Ala Met Cys Tyr Ser Ala Leu Val Thr Lys Thr Asn Arg Ile>
  a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

           2056           2066           2076           2086
       *       *       *       *       *       *       *       *
GCA CGC ATC CTG GCT GGC AGC AAG AAG AAG ATC TGC ACC CGG AAG CCC
Ala Arg Ile Leu Ala Gly Ser Lys Lys Lys Ile Cys Thr Arg Lys Pro>
  a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

2096           2106           2116           2126           2136
       *       *       *       *       *       *       *       *       *       *
AGG TTC ATG AGT GCC TGG GCT CAG GTG ATC ATT GCC TCA ATT CTG ATT
Arg Phe Met Ser Ala Trp Ala Gln Val Ile Ile Ala Ser Ile Leu Ile>
  a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

   2146           2156           2166           2176           2186
       *       *       *       *       *       *       *       *       *
AGT GTG CAA CTA ACC CTG GTG GTA ACC CTG ATC ATC ATG GAA CCC CCT
Ser Val Gln Leu Thr Leu Val Val Thr Leu Ile Ile Met Glu Pro Pro>
  a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

     2196           2206           2216           2226           2236
       *       *       *       *       *       *       *       *       *       *
ATG CCC ATT CTG TCC TAC CCA AGT ATC AAG GAA GTC TAC CTT ATC TGC
Met Pro Ile Leu Ser Tyr Pro Ser Ile Lys Glu Val Tyr Leu Ile Cys>
  a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

        2246           2256           2266           2276           2286
       *       *       *       *       *       *       *       *       *       *
AAT ACC AGC AAC CTG GGT GTG GTG GCC CCT TTG GGC TAC AAT GGA CTC
Asn Thr Ser Asn Leu Gly Val Val Ala Pro Leu Gly Tyr Asn Gly Leu>
  a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

           2296           2306           2316           2326
       *       *       *       *       *       *       *       *
CTC ATC ATG AGC TGT ACC TAC TAT GCC TTC AAG ACC CGC AAC GTG CCC
Leu Ile Met Ser Cys Thr Tyr Tyr Ala Phe Lys Thr Arg Asn Val Pro>
  a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

2336           2346           2356           2366           2376
       *       *       *       *       *       *       *       *       *       *
GCC AAC TTC AAC GAG GCC AAA TAT ATC GCG TTC ACC ATG TAC ACC ACC
Ala Asn Phe Asn Glu Ala Lys Tyr Ile Ala Phe Thr Met Tyr Thr Thr>
  a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

   2386           2396           2406           2416           2426
       *       *       *       *       *       *       *       *       *
TGT ATC ATC TGG CTA GCT TTT GTG CCC ATT TAC TTT GGG AGC AAC TAC
Cys Ile Ile Trp Leu Ala Phe Val Pro Ile Tyr Phe Gly Ser Asn Tyr>
  a   a   a   a   TRANSLATION OF PHCH4 [A]   a   a   a   a   >

     2436           2446           2456           2466           2476
       *       *       *       *       *       *       *       *       *       *
```

FIG. 5f

```
AAG ATC ATC ACA ACT TGC TTT GCA GTG AGT CTC AGT GTA ACA GTG GCT
Lys Ile Ile Thr Thr Cys Phe Ala Val Ser Leu Ser Val Thr Val Ala>
   a   a   a   a   TRANSLATION OF PHCH4 [A]    a   a   a   a   >

       2486      2496       2506       2516       2526
   *    *    *    *    *    *    *    *    *    *
CTG GGG TGC ATG TTC ACT CCC AAG ATG TAC ATC ATT ATT GCC AAG CCT
Leu Gly Cys Met Phe Thr Pro Lys Met Tyr Ile Ile Ile Ala Lys Pro>
   a   a   a   a   TRANSLATION OF PHCH4 [A]    a   a   a   a   >

         2536       2546       2556       2566
     *    *    *    *    *    *    *    *    *
GAG AGG AAT ACC ATC GAG GAG GTG CGT TGC AGC ACC GCA GCT CAC GCT
Glu Arg Asn Thr Ile Glu Glu Val Arg Cys Ser Thr Ala Ala His Ala>
   a   a   a   a   TRANSLATION OF PHCH4 [A]    a   a   a   a   >

2576      2586       2596       2606       2616
  *    *    *    *    *    *    *    *    *    *
TTC AAG GTG GCT GCC CGG GCC ACG CTG CGC CGC AGC AAC GTC TCC CGC
Phe Lys Val Ala Ala Arg Ala Thr Leu Arg Arg Ser Asn Val Ser Arg>
   a   a   a   a   TRANSLATION OF PHCH4 [A]    a   a   a   a   >

  2626       2636       2646       2656       2666
     *    *    *    *    *    *    *    *    *
AAG CGG TCC AGC AGC CTT GGA GGC TCC ACG GGA TCC ACC CCC TCC TCC
Lys Arg Ser Ser Ser Leu Gly Gly Ser Thr Gly Ser Thr Pro Ser Ser>
   a   a   a   a   TRANSLATION OF PHCH4 [A]    a   a   a   a   >

    2676       2686       2696       2706       2716
 *    *    *    *    *    *    *    *    *    *
TCC ATC AGC AGC AAG AGC AAC AGC GAA GAC CCA TTC CCA CAG CCC GAG
Ser Ile Ser Ser Lys Ser Asn Ser Glu Asp Pro Phe Pro Gln Pro Glu>
   a   a   a   a   TRANSLATION OF PHCH4 [A]    a   a   a   a   >

       2726       2736       2746       2756       2766
   *    *    *    *    *    *    *    *    *    *
AGG CAG AAG CAG CAG CAG CCG CTG GCC CTA ACC CAG CAA GAG CAG CAG
Arg Gln Lys Gln Gln Gln Pro Leu Ala Leu Thr Gln Gln Glu Gln Gln>
   a   a   a   a   TRANSLATION OF PHCH4 [A]    a   a   a   a   >

         2776       2786       2796       2806
     *    *    *    *    *    *    *    *    *
CAG CAG CCC CTG ACC CTC CCA CAG CAG CAA CGA TCT CAG CAG CAG CCC
Gln Gln Pro Leu Thr Leu Pro Gln Gln Gln Arg Ser Gln Gln Gln Pro>
   a   a   a   a   TRANSLATION OF PHCH4 [A]    a   a   a   a   >

2816      2826       2836       2846       2856
  *    *    *    *    *    *    *    *    *    *
AGA TGC AAG CAG AAG GTC ATC TTT GGC AGC GGC ACG GTC ACC TTC TCA
Arg Cys Lys Gln Lys Val Ile Phe Gly Ser Gly Thr Val Thr Phe Ser>
   a   a   a   a   TRANSLATION OF PHCH4 [A]    a   a   a   a   >

  2866       2876       2886       2896       2906
     *    *    *    *    *    *    *    *    *
CTG AGC TTT GAT GAG CCT CAG AAG AAC GCC ATG GCC CAC GGG AAT TCT
Leu Ser Phe Asp Glu Pro Gln Lys Asn Ala Met Ala His Gly Asn Ser>
   a   a   a   a   TRANSLATION OF PHCH4 [A]    a   a   a   a   >

    2916       2926       2936       2946       2956
 *    *    *    *    *    *    *    *    *    *
ACG CAC CAG AAC TCC CTG GAG GCC CAG AAA AGC AGC GAT ACG CTG ACC
Thr His Gln Asn Ser Leu Glu Ala Gln Lys Ser Ser Asp Thr Leu Thr>
```

FIG. 5g.

```
    a   a   a   a   TRANSLATION OF PHCH4 [A]    a   a   a   a   >

       2966        2976         2986        2996         3006
   *     *     *     *     *     *     *     *     *     *
CGA CAC CAG CCA TTA CTC CCG CTG CAG TGC GGG GAA ACG GAC TTA GAT
Arg His Gln Pro Leu Leu Pro Leu Gln Cys Gly Glu Thr Asp Leu Asp>
    a   a   a   a   TRANSLATION OF PHCH4 [A]    a   a   a   a   >

         3016        3026        3036         3046
       *     *     *     *     *     *     *     *     *
CTG ACC GTC CAG GAA ACA GGT CTG CAA GGA CCT GTG GGT GGA GAC CAG
Leu Thr Val Gln Glu Thr Gly Leu Gln Gly Pro Val Gly Gly Asp Gln>
    a   a   a   a   TRANSLATION OF PHCH4 [A]    a   a   a   a   >

3056        3066         3076        3086         3096
  *     *     *     *     *     *     *     *     *     *
CGG CCA GAG GTG GAG GAC CCT GAA GAG TTG TCC CCA GCA CTT GTA GTG
Arg Pro Glu Val Glu Asp Pro Glu Glu Leu Ser Pro Ala Leu Val Val>
    a   a   a   a   TRANSLATION OF PHCH4 [A]    a   a   a   a   >

  3106        3116         3126         3136        3146
    *     *     *     *     *     *     *     *     *
TCC AGT TCA CAG AGC TTT GTC ATC AGT GGT GGA GGC AGC ACT GTT ACA
Ser Ser Ser Gln Ser Phe Val Ile Ser Gly Gly Gly Ser Thr Val Thr>
    a   a   a   a   TRANSLATION OF PHCH4 [A]    a   a   a   a   >

    3156        3166        3176        3186
 *     *     *     *     *     *     *     *     *
GAA AAC GTA GTG AAT TCA T AAAATGG AAGGAGAAGA CTGGGCTAG
Glu Asn Val Val Asn Ser Xxx>
    TRANSLATION OF PHC   a >
```

FIG. 6.

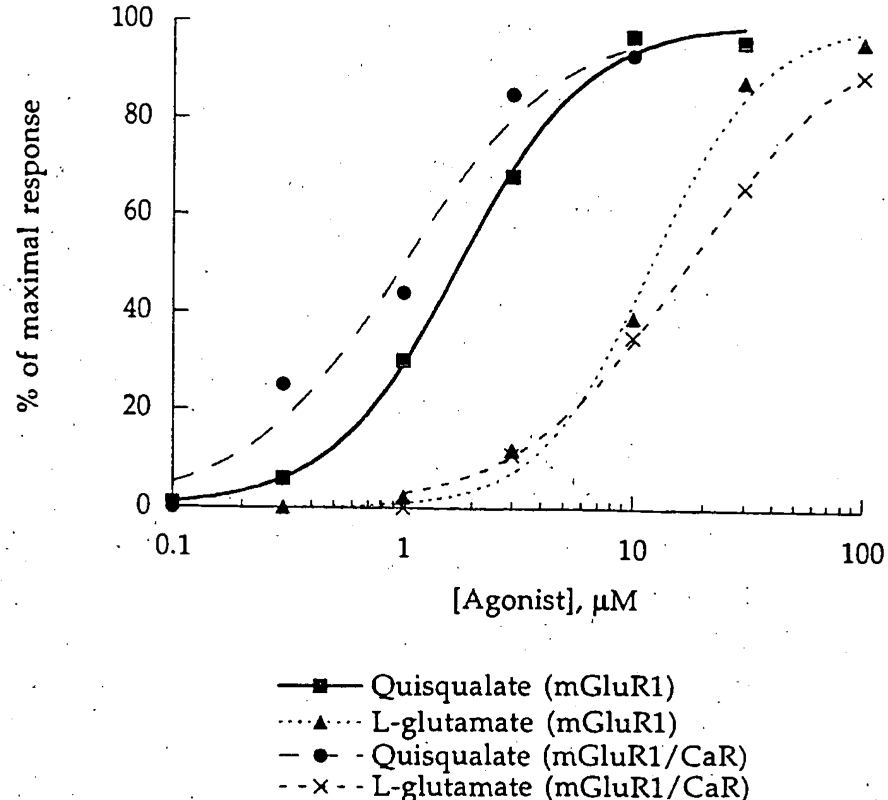

100
80
60
40
20
0
% of maximal response
0.1
1
10
100
[Agonist], µM
Quisqualate (mGluR1)
L-glutamate (mGluR1)
Quisqualate (mGluR1/CaR)
L-glutamate (mGluR1/CaR)

FIG. 7.

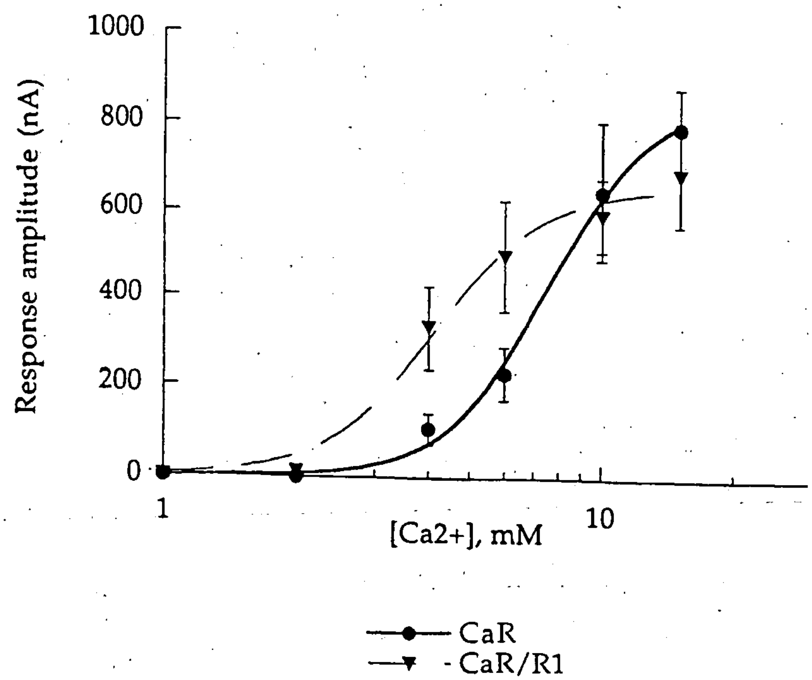

1000
800
600
400
200
0
Response amplitude (nA)
1
10
[Ca2+], mM
CaR
CaR/R1

FIG. 8a.

a) pmGluR1

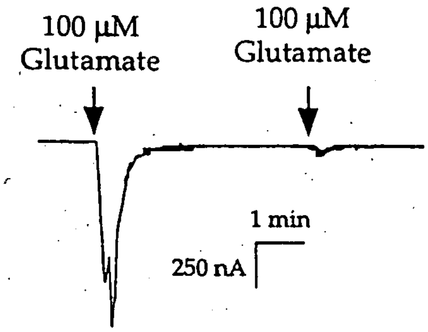


FIG. 8b.

b) hCaR

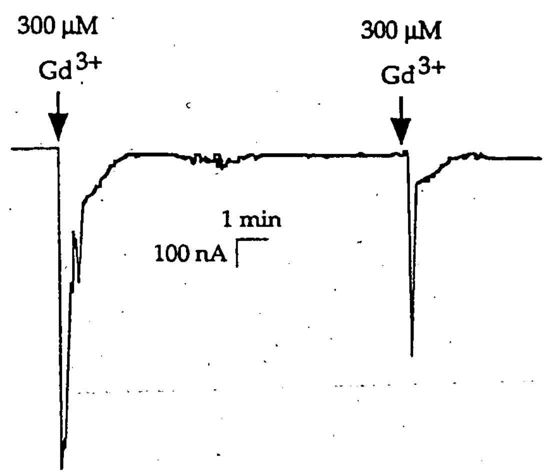


FIG. 8c.

c) pCH3

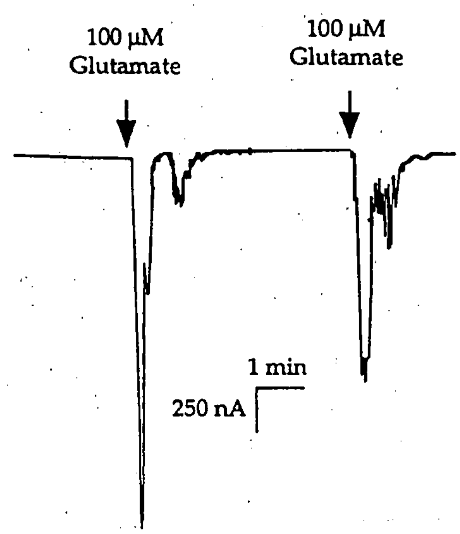
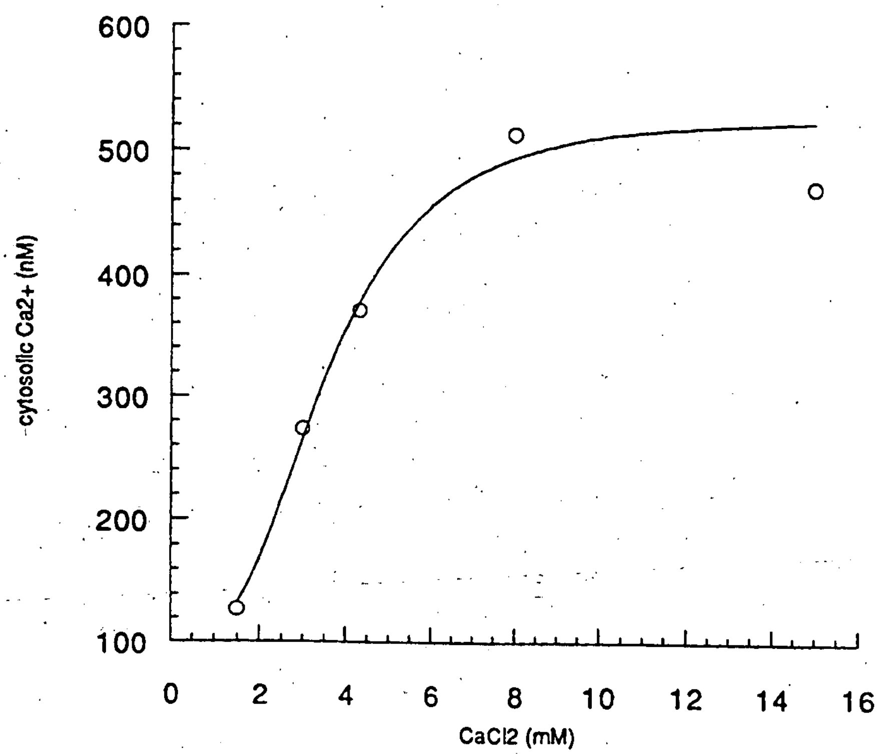


FIG. 9.



y = (m1-99.8)/(1+(m2/m0)^m3)...		
	Value	Error
m1	529.94	26.745
m2	3.5223	0.30124
m3	2.9298	0.63546
Chisq	5476.2	NA
R	0.98433	NA